

Centrifugal-Driven, Reduced-Dimension,
Planar Chromatography and Nanoscribe Mesh
Filters for Separations

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Abstract

The ability to separate chemicals is vitally useful to a wide variety of fields including chemistry, biology, pharmacology, and environmental analysis. Thin-layer chromatography is advantageous in the world of chemical separations as it is easy to use, can accommodate multiple samples at once, and has a wide range of applicability. However, this technique can be limited by band broadening, thus decreasing its efficiency. In an effort to increase efficiency particle sizes have been reduced, which in turn has decreased the mobile phase velocity. The use of micro- and nanopillar arrays systems mitigates this decrease due to the more ordered arrangement of the pillars, but efficiency is still limited by the mobile phase velocity. The work presented herein focuses on the fabrication and development of separation platforms that improve efficiency of pillar array chromatography systems by increasing mobile phase flow velocity through the use of centrifugal force.

Likewise, the ability to separate particles on the micro- and nanoscale is important for many applications such as food processing, medical diagnostics, and cosmetics. There are a variety of techniques to create devices capable of sorting and separating micro- and nanoparticles. However, these devices are aimed at separating low volume high value samples. The second project described in the work herein proposed the use of micro 3D laser printing to create mesh filters in channels for the separation of nanoparticles suspended in solutions of low volume, as well as a system allowing the study of diffusion of particles through the mesh filters.

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Abbreviations and Symbols

TLC	thin layer chromatography
HPTLC	high-performance thin layer chromatography
UTLC	ultra-thin layer chromatography
R_f	retardation factor
s_f	distance the solvent front travels
k	proportionality constant
t	time
K_0	permeability constant
d_p	particle diameter
γ	surface tension of the mobile phase
θ	contact angle of the mobile phase
μ_f	solvent front velocity
η	mobile phase viscosity
μ_f	velocity of the solvent front
Z_s	distance the sample traveled
Z_f	distance the solvent front traveled
H	plate height
A	eddy diffusion
B	molecular diffusion
D_m	diffusion coefficient of the mobile phase
μ	average linear mobile phase velocity
C_s	resistance to mass transfer in the stationary phase
d_f	film thickness of the stationary phase
C_m	resistance to mass transfer in the mobile phase
w_f	final spot width
w_i	initial spot width
d	distance the spot traveled
RIE	reactive ion etching
PECVD	plasma enhanced chemical vapor deposition

ICP	inductively coupled plasma
DRIE	deep reactive ion etching
EB-PVD	electron beam physical vapor deposition
ALD	atomic layer deposition
PSO	porous silicon dioxide
CF	centrifugal force
DW	metal dewetting
PL	photolithography
CAD	computer aided design
C4	n-butyldimethylchlorosilane
γ	independent factor specific to packing quality
ω	independent factor specific to packing quality
v	linear flow velocity
m	solvent mass distribution
V	angular velocity
S_f	radial position
ρ	solvent density
R	rotational rate (rotations per minute)
ACN	acetonitrile
v_{opt}	optimum flow velocity
A	channel cross section (adjusted for porosity)
Φ	experimental flow resistance parameter
PDMS	polydimethylsiloxane
HPLC	high performance liquid chromatography
CFD	capillary flow device
nm	nanometer
μm	micron
DD	diffusion device
SEM	scanning electron microscope
LOC	lab-on-a-chip

Chapter 1

Introduction to Planar Chromatography and Size- Based Chip Separations

1.1 –Introduction

The goal of a separation process is to segregate components of a mixture, in their pure form, into separate containers or regions. Most methods of separation involve two phases; in chromatography they are the mobile and stationary phase. All separation techniques are based on a differential phase distribution of analytes in a sample, and separations may be carried out based on difference in the states, phases, or environments.[1]

Separation techniques based on changes in the actual state of the sample are equilibrium processes involving the distribution of analyte between liquid and solid states, such as precipitation. Other such methods include distillation, sublimation, crystallization, and refining. Techniques that separate due to changes in phase distribution, wherein the sample components are dilute compounds in the phases, are also equilibrium processes and include chromatographic methods. In so far as analytical chemistry is concerned, chromatographic methods are the most powerful separation techniques available and the most used. Planar chromatography, used herein, is one such type of separation technique and will be described in further detail in this chapter. By contrast, separation methods based on environments are nonequilibrium processes and depend on different rates of migration influenced by an external force or field. These methods include field-flow fractionation, filtration, thermal diffusion, and dialysis, to name a few.[1] The sized based separations described in this dissertation fall under this technique.

1.2 – Thin Layer Chromatography

Thin layer chromatography (TLC) is a form of planar chromatography and one of the most popular and extensively used separation techniques; this is due to its ease of use, high detection sensitivity, rapid separation times, ability to accommodate a wide range of different samples, and

ability separate multiple samples at once. TLC is often used to confirm reaction completion, sample purity, and determine amounts or components of a mixture.[2]

Thin layer chromatography, first developed in 1889, is an analytical method that is still used today. In its original format, Martinus Beyerinck observed a mixture of sulfuric and hydrochloric acid diffuse through a thin layer of gelatin spread on a glass plate using visualizing agents of silver nitrate and barium chloride to detect the acids. This was the first description of planar chromatography carried out on a stationary phase other than paper.[3] It was not until 1938, when Izmailov and Shraiber reported TLC by calling it the “drop-chromatographic method”, that TLC began to resemble the powerful separation technique we are familiar with today. They described carrying out separations on stationary phases made of thin layers of lime, aluminum oxide, or magnesium oxide coated on glass plates.[2, 4] However, in the 1950’s TLC began to gain notability as an effective separation technique due to the work of Stahl and Kirchner who standardized the technique, combining the advantages of paper and column chromatography to improve reproducibility and performance.[5]

TLC as performed today consists of thin layers of a stationary phase (or sorbent material such as alumina) applied to a solid support such as a glass or plastic plate. The sample mixture is applied or spotted at the base of the plate, after which the plate is enclosed in a saturated development chamber with a small reservoir of mobile phase as seen in Figure 1.1.1. After the plate is placed into the well of mobile phase, “development” occurs as the mobile phase travels up the plate by capillary action. Separation occurs based on the distribution of the sample components between the mobile and stationary phases. Components with a greater affinity for the stationary phase will spend more time in it and therefore be more retained, i.e. not travel as far. After the mobile phase has traveled a selected distance, the plate is removed from the chamber and allowed to

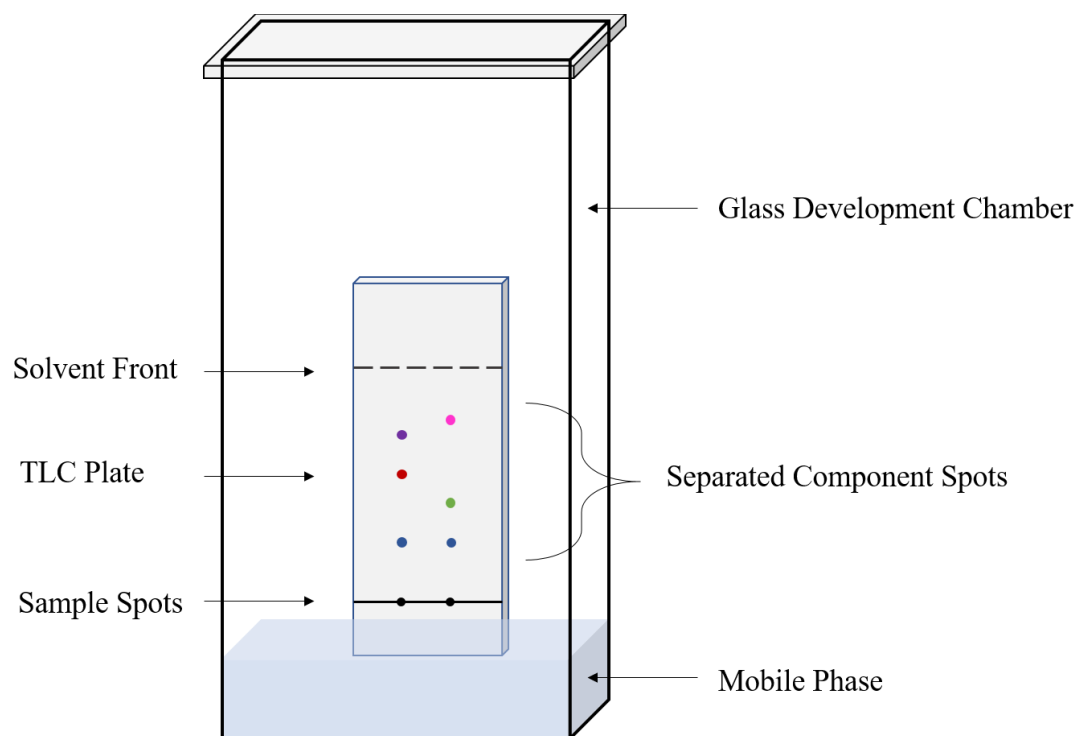


Figure 1.1.1: Typical TLC separation.

dry. After the plate has dried, the individual sample components or spots may be visualized by fluorescence, spraying with a reagent, or under an ultraviolet light.

Chromatography theory predicts that efficiency and speed of separation will increase with smaller stationary phase particle size. In traditional TLC, particle sizes range 10-12 μm with layer thickness 250-1000 μm . As more recent developments in TLC have been aimed to improve the efficiency of the technique by reducing particle size, high-performance thin layer chromatography (HPTLC) and ultra-thin layer chromatography (UTLC) have been developed. HPTLC uses stationary phases 100-250 μm thick with particles sized 5-6 μm . It is an improvement over traditional TLC as it exhibits faster development time and shorter development distances. HPTLC typically uses methods for automated sample application and analysis.[6] Micro machining methods have been used to further reduce particle size and layer thickness in the creation of stationary phases for ultra-thin layer chromatography. Chromatographic theory also predicts that velocity will decrease as particle size decreases, thereby reducing efficiency. To overcome this problem with efficiency Chapters 3 and 4 presented herein continues the research into improving chromatographic efficiency by implementing centrifugal force to increase velocity.

1.3 – Stationary Phases

Stationary phases are classified as normal phase or reverse phase: a normal phase system has a hydrophilic or polar stationary and requires relatively nonpolar solvents for the mobile phase, whereas a reverse phase system is the opposite, and has a hydrophobic or nonpolar stationary phase and requires relatively polar solvents for the mobile phase.

The most widely used stationary phases include silica gel and aluminum oxide (alumina). These are inorganic normal stationary phases that can be modified with organic silanes to become reverse stationary phases. Normal phases rely on polarity to separate sample components and can

have difficulty separating components with similar polarities; however, by reversing the stationary phase with organic silanes, selectivity between components of similar polarity can be achieved.

Traditional TLC stationary phases have particle sizes 10-12 microns and thicknesses of 250 microns. Theory predicts chromatographic efficiency will increase as particle size decreases. Developments in TLC have been aimed to reduce the particle size of the stationary phase. HPTLC is characterized by particle sizes 5-6 microns with overall thickness of approximately 150 microns. HPTLC with smaller particle sizes and phase thicknesses has reduced the required development distance by half and the development time from 30 minutes for traditional TLC to less than 10 minutes, as well as reduced the amount of sample required for a separation.

Particle size and surface area are the major factors in the effectiveness of a separation. A smaller particle size increases efficiency as given by the van Deemter equation shown later in equation [1.5.1], and a large surface area provides more sites for analyte interaction, which mitigates overloading, and increasing separation efficiency via increase in retention.[1] Advances in planar chromatography involve development of UTLC stationary phases where methods of micro-machining have been explored to improve separation efficiencies.

1.4 – Mobile Phases

The types of mobile phase can vary widely, and may be either a single solvent or a mixture of solvents. Solvents for separations are selected based on how they interact with the analytes of interest, as well as how they move through the stationary phase. It is important that they have a limited affinity for the sample components, as well as wet the stationary phase. The ideal mobile phase will create a retardation factor (R_f , discussed in 1.5) between 0.3 and 0.7.[1, 2] When all of the components of a mixture have a strong affinity for the mobile phase it not be retained on the stationary phase and will travel with the solvent front therefore not creating a separation.

Additionally, solvent mixtures used for the mobile phases should be composed of compounds that have unique functionalities yet are still miscible. In a typical normal phase system, the mobile phase would be comprised of a nonpolar organic solvent base, often a hydrocarbon, modified with a polar organic solvent such as an ester or alcohol; by contrast, in a reverse phase system, water would most frequently be the base solvent modified with a polar organic solvent such as acetonitrile or methanol.[1] Mobile phases are chosen by comparing solvent strengths and reviewing literature then refined empirically.

The flow of the mobile phase is not externally influenced but is dependent on the viscosity, η , and surface tension, γ , of the mobile phase solvents, as well as the morphology and chemical nature of the stationary phase. The mobile phase is applied to one end of the dry stationary phase planar bed and drawn up the TLC plate by capillary action. Capillary action is defined as the movement of a fluid within a capillary, a narrow tube, due to the forces of cohesion and adhesion. Although, the flow is generally against gravity, the effect of gravity at the micro- and nanoscale is negligible and movement is driven by adhesive intermolecular forces between the solvent and the substrate, as well as cohesive intermolecular forces between like-solvent compounds. Thus, the mobile phase velocity is greatest at the start of the movement of the solvent front and slows later due to increased mass and the additive effects of viscous resistance to flow. Homogeneous stationary phases and low viscosity solvents can minimize the resistance to mass flow by creating cohesive channels; however, the wicking front will still slow with development distance. The distance the solvent front travels, s_f , over time, t , is calculated:

$$s_f = \sqrt{kt} \quad [1.3.1]$$

The proportionality constant, k , is calculated:

$$k = \frac{2K_0 d_p \gamma}{\eta \cos \theta} \quad [1.3.2]$$

In this equation, K_0 is the permeability constant, d_p is the particle diameter, and θ is the contact angle of the mobile phase on the stationary phase (in TLC, θ is almost always 0). The solvent front velocity, μ , may be calculated:

$$\mu_f = \frac{k}{2s_f} \quad [1.3.3]$$

The velocity of the solvent front is directly related to surface tension and inversely related to the distance moved and viscosity. Therefore, the solvent front velocity is not constant and decreases with time.[1]

1.5 – Sample Application, Development, Detection, and Evaluation

Sample application is vastly important in the chromatographic process, and technique can have an effect on resolution and quantitation. Ideally, the sample should be applied to the smallest area possible and above the line of mobile phase immersion. Sample size is critical, as spots too high in analyte concentration can result in tailing. Tailing can cause individual bands to overlap and not completely resolve. Additionally, large amounts of the analyte may clog the stationary phase preventing the mobile phase from wicking. Samples are often applied via the plate contact method with the use of capillary tubes, micropipets, or microsyringes. For increased reproducibility and quantitation, exact volumes may be applied using commercially available automatic samplers via the spray on method or the contact spotting method.[1] For the work presented herein, samples were applied using a contact transfer method. A microsyringe and camera as seen in Figure 1.4.1

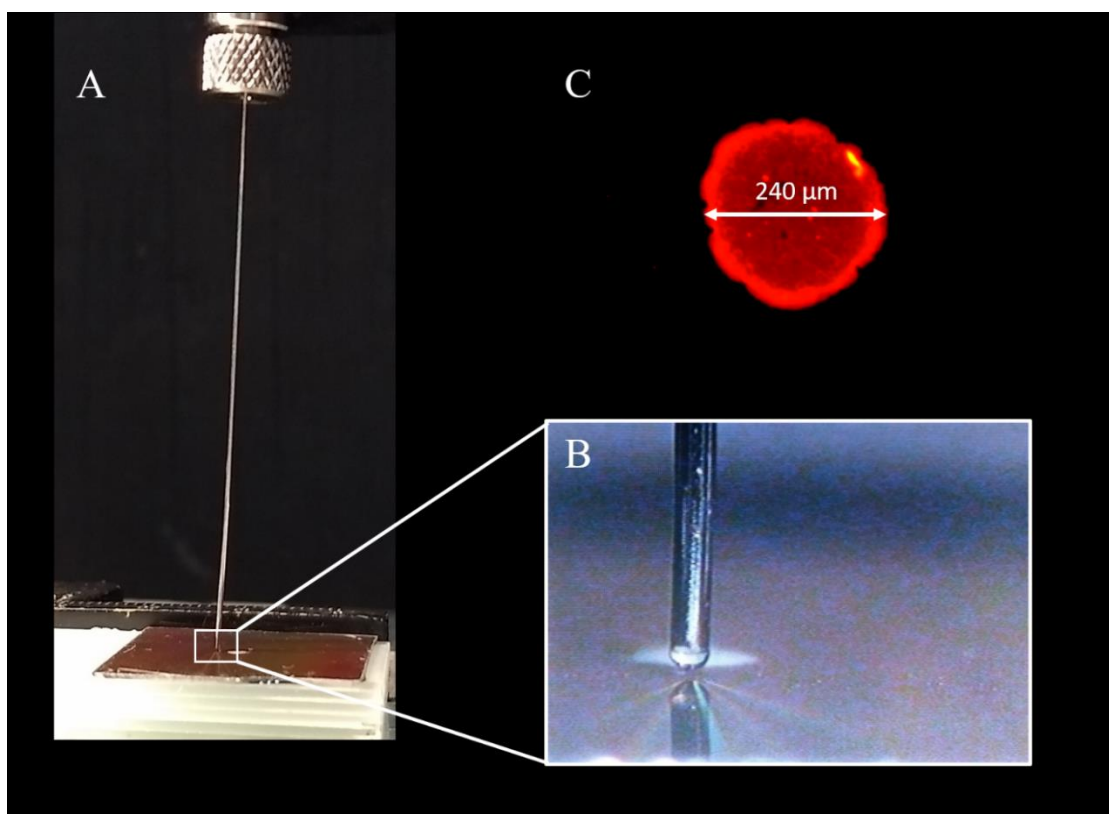


Figure 1.4.1: Depiction of the contact transfer method used to apply sample spots: (A) microsyringe spotting on pillar array. (B) CCD camera image of spotting. (C) Sample spot.

were used to precisely spot reproducible volumes. This method took advantage of the hydrophobic nature of the reverse stationary phase used and enabled small, precisely controlled, sample application.[7]

After the applied sample has dried, the plate is developed. Often this is carried out in a glass chamber saturated with the vapor of the mobile phase. A saturated development chamber allows for optimization of the chromatographic process and increases reproducibility, which can be poor in planar chromatography.[4] Most TLC development is linear in either vertical or horizontal chambers, in which the mobile phase is introduced at one end of the plate and moves to the opposite end. Development may also be circular through the use of centripetal or centrifugal development. Centripetal development is carried out when the mobile phase is applied at the edges of the plate and moves towards the center. In centrifugal development, the sample is applied around the middle of the plate; the mobile phase is then introduced to the center, and development moves from the center to the edges of the plate. The work described herein uses centrifugal development while spinning the plate to help drive the flow of the mobile phase via centrifugal forces. This forced flow technique can lead to better resolution as the optimum mobile phase velocity may be achieved (discussed in Chapter 3).[8]

Once development is complete and the plate is dry, the sample bands must be detected. For samples that fluoresce, a UV lamp is often employed detection. For non-fluorescing samples the sample bands may be located either optically, for samples in the visible range, or by quenching a phosphor in the TLC plate.[1] Developed plates may also be sprayed or dipped in universal or selective reagents to detect sample bands. TLC has been coupled with instrumental techniques such as Fourier transform infrared spectroscopy, surface enhanced Raman spectroscopy, and mass

spectrometry for detection and quantitation.[7, 9-11] The work described in this dissertation utilized a fluorescent microscope for sample detection.

After the bands have been detected, evaluation of the separation can be calculated based on the retardation factor given by:[1]

$$R_F = \frac{Z_S}{Z_F} \quad [1.4.1]$$

The R_F value is a ratio of the distance the sample traveled, Z_S , to the distance the solvent front traveled, Z_F , from the original spot. The retardation factors are dependent on the properties of the separated sample at constant temperature. Accurate and reproducible R_F values are important for sample identification. R_F values fall between 1.0, indicating the sample was not retained and traveled with the solvent front, and 0.0, in which the sample was completely retained and did not travel from the original spot. Since optimal resolution is obtained in the middle third of the plate due to more consistent phase ratios, experimental parameters should be chosen so the R_F values are between 0.3 and 0.7.[2, 12]

1.6 – Efficiency, van Deemter, and Band Broadening

Efficiency (i.e. the size of the developed sample spots) is controlled by physical parameters of the system, including the size and uniformity of the stationary phase, as well as movement of the mobile phase. To maximize efficiency, the thickness and particle size of the stationary phase, along with diffusion of the spotted sample within the mobile phase, need to be minimized. Plate height, H , is a measure of efficiency. Small H values are congruent with narrow sample bands and high efficiency. The influence of these factors may be seen in the van Deemter equation [1.5.1] as a function of average linear mobile phase velocity, μ .[2]

$$H = A(d_p) + \frac{B(D_M)}{\mu} + \left[C_s(d_f^2) + C_m \left(\frac{d_p^2}{D_M} \right) \right] \mu \quad [1.5.1]$$

The A , B , and C terms represent different types of band broadening that influence efficiency. Plate height is dependent on eddy diffusion (A), molecular diffusion (B), and resistance to mass transfer in both the mobile (C_m), and stationary phase (C_s). The B and C terms also contain a mobile phase velocity component. The A , B , and C are influenced most by the parenthetical terms particle diameter, d_p , diffusion of the mobile phase, D_M , and film thickness of the stationary phase, d_f .

The A term, eddy diffusion, results in band broadening as molecules starting at the same position take different paths through the stationary phase. The molecule taking the most direct path will travel more quickly than the molecule taking a path divergent from the linear path, resulting in band broadening. Using small particles helps to minimize band broadening due to eddy diffusion. Molecular diffusion, B , causes band dispersion as sample molecules diffuse in all directions within the mobile phase spreading from higher concentration to lower concentration as a function of time.[4] Therefore the more time the sample spends traveling along the TLC plate, the broader the resulting peak. The B term is best reduced by increasing the velocity of the mobile phase.

The C_s term, resistance to mass transfer in the stationary phase, deals with band broadening caused by delays during the sorption and desorption of the sample molecules into and out of the stationary and mobile phases. Band broadening due to the mass transfer resistance in the stationary phase occurs when some sample molecules are adsorbed deeper into the stationary phase and take more time to desorb back into the mobile phase. During that time solute molecules in the mobile phase move ahead of the sorbed molecules, causing dispersion of the band. The C_s term is therefore minimized by reducing the thickness of the stationary phase.

The C_m term, resistance to mass transfer in the mobile phase, is caused by the variations in the velocity as the mobile phase travels up the plate. Since the mobile phase moves faster in some places, sample molecules that spend more time in the faster moving zones will leave behind other

molecules traveling in the slower moving regions, resulting in band broadening. The C_m term is minimized by decreasing the gap between particles, which in traditional TLC is determined by particle size. However, when the distance between the particles is lessened by decreasing the particle size, the mobile phase velocity is reduced. By replacing particles with pillar arrays, the gap between pillars can be controlled independently of pillar size. Studies in our group on decreasing the gap between pillars have concluded that smaller gap sizes increase the efficiency of the system.[13]

While efficiency is determined by the van Deemter equation [1.5.1], experimentally it is calculated:

$$H = \frac{(W_f - W_i)^2}{16d} \quad [1.5.2]$$

Where W_f and W_i are the final and initial spot widths measured in the direction of flow and d is the distance the spot traveled. Efficiencies for the separations performed herein were calculated using equation [1.5.2].[14]

1.7 – Ultra-Thin Layer Chromatography

Studies have been devoted to reducing the size of the planar chromatography systems from TLC down to the micro- and nanoscale, to further the research in the field.[15-17] Fabrication processes from the semiconductor industry have been modified to develop micro- and nano-structure on chip separation systems.[18] Fabrication of such systems use cleanroom processing techniques such as photolithographic patterning, thermal dewetting of thin platinum films, reactive ion etching (RIE) of silicon, and plasma enhanced chemical vapor deposition (PECVD) of silicon oxides (discussed in Chapter 2).[19-21]

Advances in planar chromatography involve development of UTLC stationary phases, where methods of micro-machining have been explored to improve separation efficiencies through reduction in the size of the separation bed features. Work done by Saha, Brett, and Olesik has been significant in the development of ultra-thin stationary phases. Brett et. al. used glancing angle deposition to deposit silicon dioxide on glass substrate to create nanostructured stationary phases.[22] Olesik et. al. used electrospinning to create nanofibrous stationary phases, which exhibited tunable retention and improved efficiencies over commercial plates.[23, 24] Saha et. al. investigated the relationship between capillary flow and pillar diameter, pitch, and height in microchannels with fabricated pillars.[25]

Particle size and surface area are the two most important factors in the effectiveness of a separation. A smaller particle size increases efficiency as given by the Van Deemter equation, and a large surface area provides more sites for analyte interaction, thereby improving performance.[1] However, initial attempts to improve efficiency by scaling down the system resulted in a reduction of efficiency due to an increase in the nonuniformity of the system. By contrast, efficiency was improved when lithographically fabricated pillars replaced heterogeneous and polydisperse particles. Greater efficiency is seen with these fabricated pillar arrays due to their almost perfect order and decreased flow resistance compared to traditional systems.[26] Regnier, Desmet, and Tallarek have conducted numerous studies of fluid dynamics and enclosed micromachined pillar arrays systems, which have provided motivation for exploring pillar arrays as planar chromatographic substrates.[15-17, 27-31] Our previous work with fabricated pillar arrays, another UTLC stationary phase, has shown an increase in efficiencies due to the replacement of polydisperse heterogeneous packing particles traditionally used with deterministic pillar arrays fabricated via photolithography.[18] Plate height (H) was improved due to less resistance to mass

transfer in the mobile phase (C_M) from smaller pillar diameters and interpillar gaps, less molecular diffusion band broadening from greater permeability, as well as the absence of eddy diffusion. More recently, we have further reduced the planar chromatographic platforms to the nano-dimensions using electron beam lithography and metal dewetting fabrication methods.[14] With the UTLC and nanoscale pillar arrays, the flow is significantly faster than that in traditional TLC; however, molecular diffusion still limits efficiency during development.[14, 18]

1.8 – Size Separations

Micro- and nanoparticle separation is an important part of many microfluidic devices used for the separation of biological and synthetic samples in a variety of fields. A technique founded on the separating particles according to size is known as size exclusion chromatography. This separation technique is different from other liquid-chromatography techniques in that it is not based on chemical attractions and interactions, but upon the size of the sample molecules. This is accomplished by controlling the size of the pores in the stationary phase, thus allowing smaller molecules to pass through while larger molecules are stopped. In this technique, the mobile phase simply acts as an eluent and solvent for the sample. For the work presented herein, mesh filters were designed and fabricated to separate nanoparticles in microfluidic channels.

1.9 – Summary

TLC is one of the most popular and widely used separation techniques due to its detection sensitivity, ease of use, and multiplex ability over other separation techniques. However, it suffers from lack of efficiency and reproducibility. Typical TLC separations take approximately 30 minutes and results may vary. Nonuniform and large particle sizes of the stationary phase lead to band broadening. The nonuniformity of the stationary phases make reproducibility difficult, while

reducing particle size alone causes a reduction in mobile phase velocity, which leads to band broadening due to molecular diffusion.

The work described herein aims to improve both efficiency and reproducibility with the use of pillar arrays and centrifugal force. The use of pillar arrays decreases the particle size and increases the uniformity of the stationary phase without the drastic decreases in flow. Additionally, the use of centrifugal force adds a new element of control to the mobile phase velocity. The van Deemter equation is used to show and test the efficiency of the new nanoscale chromatographic system.

1.10 – References

- [1] Miller, J. M., *Chromatography Concepts and Contrasts*, John Wiley & Sons, Inc., New Jersey 2005.
- [2] Touchstone, J. C., *Practice of Thin-Layer Chromatography* John Wiley & Sons, Inc., New York 1992.
- [3] Sherma, J., in: Issaq, H. J. (Ed.), *A Century of Separation Science*, Marcel Dekker, Inc., New York 2001, pp. 49-68.
- [4] Spangenberg, B., Poole, C. F., Weins, C., *Quantitative Thin-Layer Chromatography: A Practical Survey*, Springer-Verlag Berlin, Berlin 2011.
- [5] Poole, C. F., *Journal of Chromatography A* 1999, 856, 399-427.
- [6] Indrayanto, G., *Analytical Aspects of High Performance Thin Layer Chromatography*, Springer-Verlag Berlin, Berlin 2011.
- [7] Wallace, R. A., Charlton, J. J., Kirchner, T. B., Lavrik, N. V., Datskos, P. G., Sepaniak, M. J., *Anal. Chem.* 2014, 86, 11819-11825.
- [8] Rabel, F., Sherma, J., *Journal of Liquid Chromatography & Related Technologies* 2017, 40, 165-176.
- [9] Sherma, J., *Journal of Aoac International* 2016, 99, 323-331.
- [10] Freye, C. E., Crane, N. A., Kirchner, T. B., Sepaniak, M. J., *Anal Chem* 2013, 85, 3991-3998.
- [11] Santiago, M., Strobel, S., in: Lorsch, J. (Ed.), *Laboratory Methods in Enzymology: Cell, Lipid and Carbohydrate*, Elsevier Academic Press Inc, San Diego 2013, pp. 303-324.
- [12] Variyar, P. S., Chatterjee, S., Sharma, A., *Fundamentals and Theory of HPTLC-Based Separation*, Springer-Verlag Berlin, Berlin 2011.
- [13] Crane, N. A., Lavrik, N. V., Sepaniak, M. J., *Analyst* 2016, 141, 1239-1245.

- [14] Kirchner, T. B., Strickhouser, R. B., Hatab, N. A., Charlton, J. J., Kravchenko, II, Lavrik, N. V., Sepaniak, M. J., *Analyst* 2015, *140*, 3347-3351.
- [15] De Malsche, W., Clicq, D., Verdoold, V., Gzil, P., Desmet, G., Gardeniers, H., *Lab on a Chip* 2007, *7*, 1705-1711.
- [16] Gzil, P., Vervoort, N., Baron, G. V., Desmet, G., *Anal. Chem.* 2003, *75*, 6244-6250.
- [17] De Smet, J., Gzil, P., Vervoort, N., Verelst, H., Baron, G. V., Desmet, G., *Anal. Chem.* 2004, *76*, 3716-3726.
- [18] Kirchner, T. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., *Anal. Chem.* 2013, *85*, 11802-11808.
- [19] Lavrik, N. V., Taylor, L. C., Sepaniak, M. J., *Lab on a Chip* 2010, *10*, 1086-1094.
- [20] Agapov, R. L., Srijanto, B., Fowler, C., Briggs, D., Lavrik, N. V., Sepaniak, M. J., *Nanotechnology* 2013, *24*, 505302.
- [21] Charlton, J. J., Lavrik, N., Bradshaw, J. A., Sepaniak, M. J., *ACS Applied Materials & Interfaces* 2014, *6*, 17894-17901.
- [22] Bezuidenhout, L. W., Brett, M. J., *Journal of Chromatography A* 2008, *1183*, 179-185.
- [23] Clark, J. E., Olesik, S. V., *Journal of Chromatography A* 2010, *1217*, 4655-4662.
- [24] Clark, J. E., Olesik, S. V., *Anal. Chem.* 2009, *81*, 4121-4129.
- [25] Saha, A. A., Mitra, S. K., Tweedie, M., Roy, S., McLaughlin, J., *Microfluid. Nanofluid.* 2009, *7*, 451-465.
- [26] Poole, S. K., Poole, C. F., *Journal of Chromatography A* 2011, *1218*, 2648-2660.
- [27] He, B., Regnier, F., *J. Pharm. Biomed. Anal.* 1998, *17*, 925-932.
- [28] Clicq, D., Tjerkstra, R. W., Gardeniers, J. G. E., van den Berg, A., Baron, G., Desmet, G., *Journal of Chromatography A* 2004, *1032*, 185-191.

- [29] Daneyko, A., Hlushkou, D., Khirevich, S., Tallarek, U., *Journal of Chromatography A* 2012, 1257, 98-115.
- [30] Daneyko, A., Khirevich, S., Holtzel, A., Seidel-Morgenstern, A., Tallarek, U., *Journal of Chromatography A* 2011, 1218, 8231-8248.
- [31] Lavrik, N. V., Taylor, L. T., Sepaniak, M. J., *Anal. Chim. Acta* 2011, 694, 6-20.

Chapter 2

Fabrication Methods

2.1 – Introduction

The ability to analytically separate substances is vital in the field of research for applications including drug development, neuroscience, cell-sorting, DNA analysis, and environmental analysis.[1, 2] Lab on a chip microfabrication technologies based on the microelectronics industry have become an increasingly popular way to produce platforms for analytical separations.[3] These techniques may be used to create a multitude of platforms for separations due to the precise controllability of the layout and diminutive nature of the features, down to the nanoscale.[1, 4-11] The work described herein uses photolithography, lithography-free, reactive ion etching, thin film depositions, and nanoscribe printing to construct separation platforms. This chapter introduces the basic techniques that are used in combination to fabricate pillar array-based UTLC stationary phases and nanoparticle separation platforms. Specific parameters used for each technique maybe found in the experimental sections of the subsequent chapters.

2.2 – Photolithography

Photolithography is the technique used to transfer a computer-generated pattern onto a substrate, such as a silicon wafer or glass plate, using a UV light source. The largest advantage of this technique is its reproducibility; however it has limited resolution due to the diffraction limit of the wavelength of light that is used. This technique is accomplished in three basic steps: making a mask, exposure, and etching/liftoff.[12]

The mask is made with a series of photographic processes using e-beam or optical pattern generators, resulting in a glass plate with the pattern in a thin chromium film. The substrate is prepared, cleaned, dehydrated, and a thin film of silicon oxide or nitride is deposited. Photoresist is then applied by spin coating. The photoresist is a polymeric light sensitive liquid material that

is applied in a thin layer (0.5-2.5 μm) to the substrate by spinning. The spinning speed and viscosity of the resist determines the final thickness. There are positive and negative photoresists. When using a positive resist, the areas that are exposed to UV light are dissolved during development; for a negative resist the opposite is true, where the areas that are not exposed to the UV light will be dissolved during subsequent development. Positive photoresists are used in the work described herein.[12]

Following the spin coating of the photoresist, the substrate is placed on a hot plate (soft baked) to drive off solvents from the resist and improve bonding to the substrate. The substrate is then exposed to UV light through the mask. There are three different modes in which the substrate can be exposed: proximity, contact, and projection. Proximity exposure is when there is a small gap between the mask and the photoresist during exposure. Contact exposure occurs when the mask is in direct contact with the photoresist when exposed, which yields better resolution than proximity but can damage the mask. With projection exposure, the mask image is projected onto the substrate using a dual lens optical system, but requires a step-and-repeat system to cover the whole substrate.[12]

After exposure, a post exposure bake is used to reduce the effect of standing waves. The standing wave effect results from exposing the resist on a highly reflective substrate, like a silicon wafer, to light of near normal incidence. Waves are produced in the resist perpendicular to the substrate. The nodes of the standing waves do not allow the underlying layers of resist to be properly exposed.[13] The post-exposure bake increases feature quality and stability by smoothing out the standing waves. The photoresist is then developed in an appropriate solvent to remove the unpolymerized resist, thus resulting in a wafer with the desired pattern.[12] A

schematic of the photolithography process may be seen in Figure 2.2.1. Photolithography was used in this work to create channels and deterministic (highly ordered) pillar arrays.

2.3 – Lithography Free Fabrication

Solid state metal dewetting is used as a lithography free approach to create nanoscale stochastic (disordered) pillar arrays for UTLC stationary phases. This process involves the physical vapor deposition of a thin platinum film onto a silicon wafer. The platinum film is then agglomerated or broken up into small islands by heating.[14] This occurs below the melting temperature, so the film stays in a solid state. The minimization of total energy associated with film's interfaces with substrate are the driving force behind solid state dewetting.[15, 16] The metal islands pattern the silicon wafer and act as mask during the etching process. Metal dewetting was used in this work to create stochastic pillar arrays.

2.4 – Reactive Ion Etching

Once the silicon wafer has been patterned by photolithography or metal dewetting etching is required to remove the undesired material, thus exposing the desired features. Inductively coupled plasma (ICP) reactive ion etching (RIE) was the etching process used in the work described herein. ICP RIE is a dry etching technique that employs a combination of chemical and physical processes. RIE is the most commonly used dry etching process and utilizes radio frequency energies to produce chemically active ions by stripping ions from the gas mixture inside the chamber. The etching mainly occurs as the ions bombard the surface of the wafer, chemically reacting with the wafer to remove silicon. The physical etching occurs as the high energy ions remove material by transfer of kinetic energy. This is highly directional process in that the vertical etch rate is higher than the horizontal. However, RIE results in two different etching Profiles: isotropic and anisotropic. Figure 2.4.1 illustrates the etching profiles and a

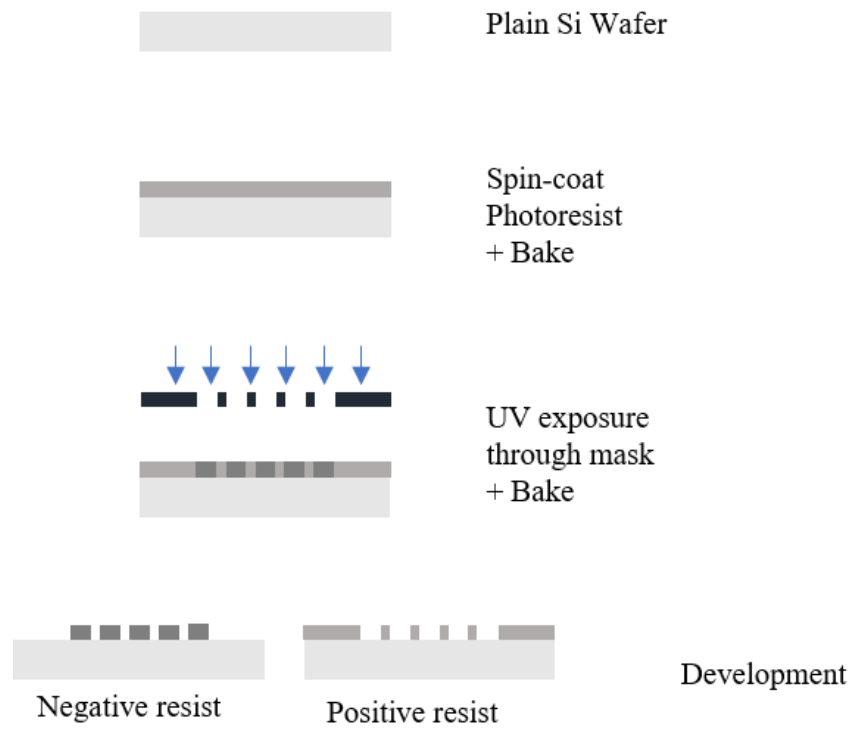


Figure 2.2.1: Schematic of the photolithography process.

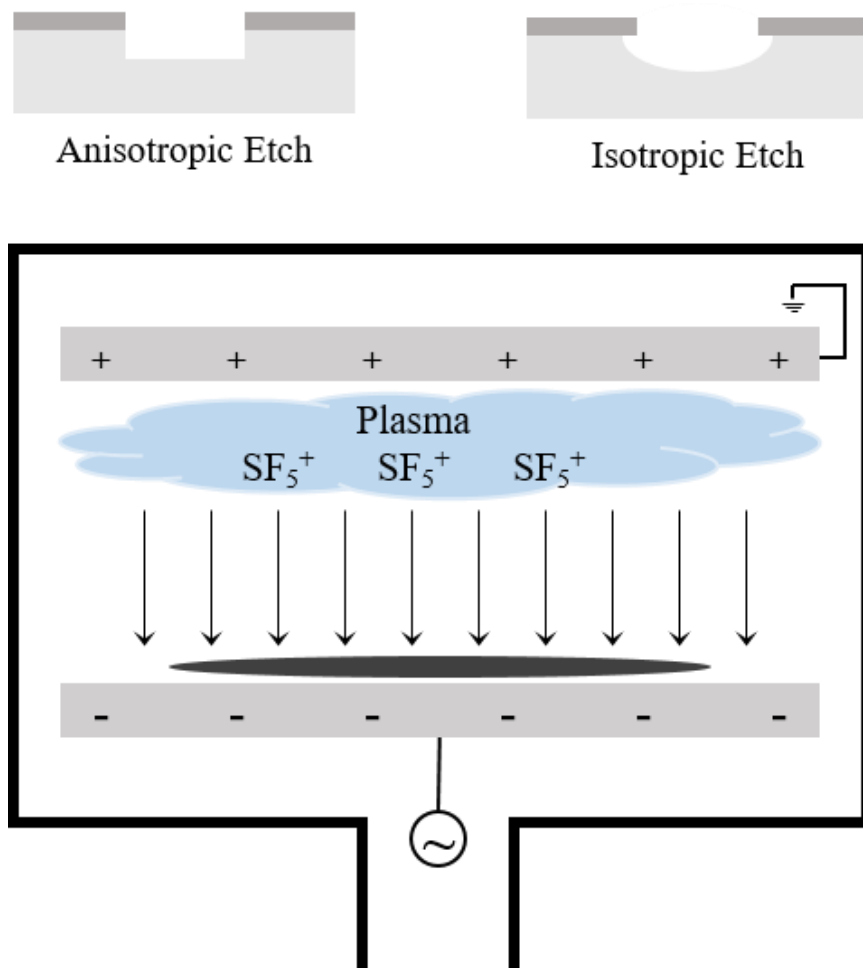


Figure 2.4.1: Illustration of isotropic and anisotropic etching profiles (top) and a reactive ion etcher reaction chamber (bottom).

general RIE reaction chamber.[17]

RIE is a useful for etching technique for silicon, but limited to a depth of 10 μm due to etch rates. However, deep reactive ion etching (DRIE) compensates for this by using a cycling two-step process of etching and passivation deposition, which allows etch depths of several hundred microns.[18] DRIE is the technique implemented in the Bosch etching recipe, which was utilized to fabricate the photolithographic pillar arrays used in this work. A schematic of the Bosch etching process and the resulting pillar may be seen in Figure 2.4.2. The Bosch process has the added benefit of increasing the surface area and stability of the pillar due to some undercutting in the etching process resulting in scalloped walls. In the first step of the DRIE Bosch process, the silicon wafer is exposed to SF_6 which isotropically etches the exposed silicon. This step is followed by the passivation deposition step, in which a C_4F_8 fluoropolymer is deposited onto the exposed surfaces. As the cycle repeats, the fluoropolymer on the bottom is etched through by the SF_6 due to its isotropic etching, while the sidewalls remain protected by the fluoropolymer. The cycle is repeated until the desired pillar height is reached.[17] Reactive ion etching was used in this work to etch pillar arrays and channels.

2.5 – Thin Film Deposition

Electron beam physical vapor deposition (EB-PVD), plasma enhanced chemical vapor deposition (PECVD), and atomic layer deposition (ALD) are methods also used in this work for thin film deposition. Electron beam physical vapor deposition was to deposit platinum, for the creation of stochastic pillars by dewetting, and gold, for coating of nanoscribe printed features. PECVD was used at low temperatures to deposit porous silicon dioxide (PSO). The PSO increases surface area, allows for pillar substrates to be functionalized with a reverse stationary phase, and increases adhesion of nanoscribe printed features. ALD was used to deposit alumina

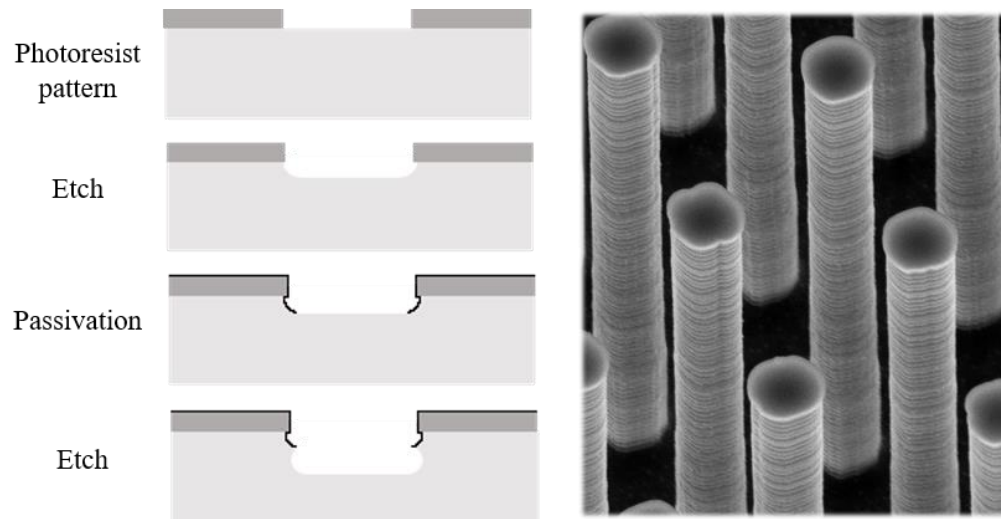


Figure 2.4.2: A schematic of the Bosch etching process (left) and the pillars (right) it creates.

and silicon oxide on substrates to increase hydrophilicity of nanoscribe features and decrease the hole size of nanoscribe meshes.

The EB-PVD process takes place inside a vacuum chamber and uses a focused high energy electron beam from an electron gun to melt and evaporate metal nuggets inside of a crucible. Deposition occurs as the evaporated metal condenses on the surface of the sample. This is a line of sight process in which the sample is placed above the crucible with the metal. Samples are often rotated during deposition to allow for a more uniform coating multiple electron guns may be used in the process.

PECVD is accomplished by using radio frequency to ionize gases. The free radicals of the gases absorb to the substrate and chemically bond to other atoms on the surface, creating a thin film. The advantage of PECVD over other chemical vapor deposition methods is that depositions may be conducted at a wider range of temperatures.[17] When deposition is carried out with silicon oxide at room temperature, the resulting film is porous.[4, 19-23]

ALD is accomplished by cycling gases that react with the substrate. Each gas-surface reaction is a half reaction and only makes up part of the material being deposited. After the first gas has reacted, that gas is purged and the second gas is introduced to the reaction chamber. The second gas completes the reaction by depositing another monolayer on the surface, after which this gas is purged and the cycle repeats until the desired film thickness is achieved. ALD differs from PECVD in that the deposition process is more conformal and takes place at temperatures around 350°C.[24]

2.6 – Nanoscribe

A Nanoscribe Pro GT laser lithography system was used to fabricate meshes in channels and micropores for particle separations, as well as central features to control flow and increase

reproducibility of centrifugal chromatographic separations. The Nanoscribe is a maskless lithography and 3D micro printing system. The process is similar to photolithography (described previously in section 2.2) and uses some of the same resists. It is a direct laser writing process and polymerizes photoresist when two photons of near-infrared light from a short laser pulse are absorbed at the same time. Computer aided design software allows for the desired features to be created. A positive photoresist is applied to a silicon or glass substrate and loaded into the instrument. An 800-nm femtosecond pulsed laser then polymerizes the photoresist by inducing a crosslinking of the polymer chains through a nonlinear two photon absorbance process. The laser focuses in the resist and polymerization occurs at the focal spot volume (voxel). Resolution is determined by the power of the laser source, laser spot size, and the properties of the photoresist. A small laser spot size may be obtained by using focusing optics with a high numerical aperture. The designed features are built up layer by layer as the sample stays fixed and the laser voxel is scanned laterally by galvanometric mirrors, while piezo actuators are used to control vertical movement. This method allows for precise control of the focal trajectory. Once the printing is complete the substrate is removed from the instrument and developed to remove unpolymersized resist, leaving the desired features behind on the substrate.[25] The Nanoscribe was used in this work to create central features and mesh filters.

2.7 – Summary

Using the fabrication methods described above, originally developed for the semiconductor industry, two different platforms for separations were created. Photolithography and metal dewetting were used to create micro- and nanoscale pillar arrays for chromatographic separations, while photolithography and nanoscribe printing were used to create substrates for

nano- and microparticle separations. Porous silicon dioxide was used in the fabrication of both substrates, increasing surface area for the pillar arrays and adhesion of the nanoscribed features.

2.8 – References

- [1] He, B., Regnier, F., *J. Pharm. Biomed. Anal.* 1998, 17, 925-932.
- [2] Saha, A. A., Mitra, S. K., Tweedie, M., Roy, S., McLaughlin, J., *Microfluid. Nanofluid.* 2009, 7, 451-465.
- [3] De Malsche, W., Clicq, D., Verdoold, V., Gzil, P., Desmet, G., Gardeniers, H., *Lab on a Chip* 2007, 7, 1705-1711.
- [4] Kirchner, T. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., *Anal. Chem.* 2013, 85, 11802-11808.
- [5] Gzil, P., Vervoort, N., Baron, G. V., Desmet, G., *Anal. Chem.* 2003, 75, 6244-6250.
- [6] Patel, R. B., Gopani, M. C., Patel, M. R., *Chromatographia* 2013, 76, 1225-1231.
- [7] Lavrik, N. V., Taylor, L. C., Sepaniak, M. J., *Lab on a Chip* 2010, 10, 1086-1094.
- [8] Lavrik, N. V., Taylor, L. T., Sepaniak, M. J., *Anal. Chim. Acta* 2011, 694, 6-20.
- [9] De Malsche, W., De Bruyne, S., De Beek, J. O., Sandra, P., Gardeniers, H., Desmet, G., Lynen, F., *Journal of Chromatography A* 2012, 1230, 41-47.
- [10] Eghbali, H., De Malsche, W., Clicq, D., Gardeniers, H., Desmet, G., *Lc Gc Europe* 2007, 20, 208-+.
- [11] De Malsche, W., Eghbali, H., Clicq, D., Vangeloooven, J., Gardeniers, H., Desmet, G., *Anal. Chem.* 2007, 79, 5915-5926.
- [12] Mack, C. A., *Fundamental principles of optical lithography : the science of microfabrication*, John Wiley & Sons, Ltd., NJ, USA 2007.
- [13] Lang, R. J., Smilowitz, B., *Ieee Transactions on Sonics and Ultrasonics* 1980, 27, 134-136.
- [14] Kaplan, W. D., Chatain, D., Wynblatt, P., Carter, W. C., *J. Mater. Sci.* 2013, 48, 5681-5717.

- [15] Thompson, C. V., *Annual Review of Materials Research* 2012, 42, 399-434.
- [16] Ye, J., *Sci Rep* 2015, 5, 9823.
- [17] *Springer Handbook of Nanotechnology*, Springer-Verlag Berlin Heidelberg, Germany 2004.
- [18] Jansen, H. V., de Boer, M. J., Unnikrishnan, S., Louwerse, M. C., Elwenspoek, M. C., *J. Micromech. Microeng.* 2009, 19, 41.
- [19] Ceiler, M. F., Kohl, P. A., Bidstrup, S. A., *J. Electrochem. Soc.* 1995, 142, 2067-2071.
- [20] Crane, N. A., Lavrik, N. V., Sepaniak, M. J., *Analyst* 2016, 141, 1239-1245.
- [21] Charlton, J. J., Jones, N. C., Wallace, R. A., Smithwick, R. W., Bradshaw, J. A., Kravchenko, II, Lavrik, N. V., Sepaniak, M. J., *Anal. Chem.* 2015, 87, 6814-6821.
- [22] Lincoln, D. R., Lavrik, N. V., Kravchenko, I. I., Sepaniak, M. J., *Anal. Chem.* 2016, 88, 8741-8748.
- [23] Charlton, J. J., Lavrik, N., Bradshaw, J. A., Sepaniak, M. J., *ACS Applied Materials & Interfaces* 2014, 6, 17894-17901.
- [24] Johnson, R. W., Hultqvist, A., Bent, S. F., *Materials Today* 2014, 17, 236-246.
- [25] NanoscribeGmbH; accessed April 16, 2018, <https://www.nanoscribe.de/en/> 2018.

Chapter 3

Centrifugal-Driven, Reduced-Dimension, Planar Chromatography

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3.1 – Abstract

A fundamental problem with efficiency in capillary action driven planar chromatography results from diminishing flow rates as development proceeds, giving rise to molecular diffusion related band dispersion for most sample types. Overpressure and electrokinetic means to speed flow have been used successfully in TLC. We explore the use of centrifugal force (CF) to drive flow for reduced-dimension planar platforms (ultra-TLC, low micrometer features, and nano-TLC, nanoscale features). The silicon wafer platforms have two forms of continuous 2-D arrays created by either photolithography or metal dewetting followed by deep reactive ion etching and coated with porous SiO₂. The flow pattern is unusual with co-planar flows above and within the arrays. The effects of parameters such as spin rate, solvent type, and surface character on flow rates are established and can be substantially greater than capillary action flow. Using fluorescent dyes, we investigate retardation factors and chromatographic plate height; the latter falls in the

low to sub-micrometer range. To the best of our knowledge, we demonstrate the first analytical separations performed in pillar arrays using CF to augment solvent flow.

3.2 – Introduction

Planar Chromatography, most commonly TLC, is a well-established separation technique with both advantages and limitations.[1,2] The advantageous features include simplicity and low cost, the ability to separate multiple samples simultaneously, no detection time constraints, and the ability to perform true orthogonal 2-dimensional separations. On the other hand, classical TLC is limited in reproducibility and efficiency due to the characteristics of capillary driven flow. Capillary forces cause the solvent to flow through the porous layer of stationary phase and against the hydraulic resistance. As the development distance of the solvent front increases, the flow resistance increases and the mobile phase velocity decreases. Since zones migrate more slowly with the evolution of the separation, band broadening eventually dominates the differential rate of migration of the zones.[3] Specifically, molecular diffusion limits efficiency, often hindering the practical use of traditional TLC in chemical separations.

Higher efficiency and shorter development distances are possible with smaller stationary phase particles and thin bed layers of high performance TLC (HPTLC).[4] HPTLC is frequently automated leading to reproducible sample application and detection for quantitative analysis.[5] However, small particles reduce flow rates so the advances are limited with HPTLC.[4,6] In recent years, ultra-TLC (UTLC) has been shown to improve efficiency while decreasing development time, sample volume, and solvent consumption compared to traditional TLC.[7] Over the last decade, UTLC plates have been introduced with a variety of stationary phases. These phases have included monolithic silica structures,[8] porous nanostructured silica films

(via glancing angle deposition),[9, 10] carbon-nanotube-templated microfabrication of porous material [11], and nano-fibrous stationary phases (prepared by electrospinning).[12]

Our previous work with fabricated silicon pillar arrays, another stationary phase for UTLC, has shown improved efficiency by replacing the relatively polydisperse and heterogeneous packing particles in traditional structures with periodic pillar arrays fabricated via photolithography (PL).[13] It was demonstrated that plate height (H) is improved due to the lack of eddy diffusion, less resistance to mass transfer in the mobile phase with smaller pillar diameters and interpillar gaps, as well as less molecular diffusion band broadening due to greater permeability. More recently, we have reduced the scale of the planar chromatographic platforms to nano-dimensions using electron beam lithography and metal dewetting (DW) fabrication methods.[14] With both the UTLC pillar arrays and the nanoscale cases the flow is considerably faster than for traditional TLC; however, efficiency is still limited during most of the development by molecular diffusion.[13, 14] Hence, we explore for the first time the use of centrifugal force, a forced-flow technique, to augment flow in reduced dimension planar chromatography.

Forced-flow techniques have been introduced to overcome the problems with capillary flow. These techniques include overpressure TLC,[15–17] planar electrochromatography,[17, 18] and rotational planar chromatography (centrifugal TLC).[16, 17, 19] In overpressure TLC the sorbent layer is covered by a thin, flexible membrane, in which the mobile phase is forced through by pressure generated from a conventional high-pressure LC pump.[16] Planar electrochromatography is a technique that is performed on a TLC plate using a large electric field as well as an aqueous buffer as a component of the mobile phase. Thus, the mobile phase moves due to electroosmotic flow.[16] In centrifugal TLC, the TLC plate is rapidly rotated and the flow

of the mobile phase is driven by CF. Elution by the mobile phase forms circular bands of the separated sample components. Centrifugal TLC has been previously used as a low efficiency preparative separation technique where the mobile phase elutes sample components off the TLC layer and into a collection vessel. The Chromatotron, a commercially available instrument for centrifugal TLC, is used for preparative separations and purifications of products.[20–24] The scale of the instrumentation is such that there is no carry over to the diminutive scale of the work herein. In contrast to its previous use, CF as an analytical separation technique for pillar arrays is examined.

3.3 – Experimental

Nanoscale stochastic pillar array fabrication

The nanoscale stochastic pillar arrays were fabricated using a lithography-free approach of DW a dual gun electron beam evaporation chamber was used to vapor deposit of a thin platinum (Pt) film (8–10 nm; Thermonics Laboratory, VE-240) on a p-type silicon wafer with 100 nm of thermally grown SiO₂. During the Pt deposition, the deposition rate and average (mass based) thickness of the deposited metal were monitored with a quartz crystal. The film was then rapidly heated to approximately 900°C in a cold wall furnace (Easy Tube 3000, First Nano, NY, USA) using a 10:1 mixture of argon and hydrogen at 735 torr. The thermally created Pt islands were subsequently used as a selective mask for anisotropic reactive ion etching (Oxford Plasma Lab, Oxford Instruments, UK) of the substrate material as described and characterized previously [25, 26].

Microscale deterministic PL pillar array fabrication

The high aspect-ratio pillar arrays were designed using CAD software. Silicon wafers (p-type, 100 mm, 300–500 μm thickness, 0.01–20 Ω resistivity) were used as the base of the arrays,

with dimensions of 1"× 1". After the spin coating and baking of a double-layer resist system (lift-off resist LOR-1A overcoated by positive tone photoresist 955 CM-2.1, Microchem), a Quintel contact aligner was used for photolithographic patterning. The contact aligner exposed the wafer to UV light through a mask with the CAD pattern. After development, the wafer was exposed to oxygen plasma for 30 s at 100 W (Oxford Reactive Ion Etching System, Oxford Instruments) to remove residual resists on the arrays. For the liftoff process, an 18 nm chromium (Cr) layer was first deposited using a dual gun electron-beam evaporation chamber. The excess resists and Cr were then removed via lift-off using an acetone bath followed by an isopropyl alcohol rinse. The wafer was then dried under a stream of nitrogen. Anisotropic deep reactive ion etching (DRIE, System 100 Plasma Etcher, Oxford Instruments) was used to form pillars 15–20 μm in height.

Porous silicon dioxide deposition

In order to increase the surface area, we added a second level of roughness to the sidewalls, floors, and tops of our pillared substrates via room temperature plasma-enhanced chemical vapor deposition of porous silicon dioxide (PSO).[27] The thin layer of PSO (25 nm) was deposited on the wafer surface using a PECVD System 100 Plasma Deposition Tool (Oxford Instruments). During the deposition of PSO, the substrate temperature and chamber pressure were 27°C and 600 mTorr, respectively. The pillar dimensions were evaluated using a scanning electron microscope (Carl Zeiss, Merlin).

Functionalization

For gas phase functionalization to form a reversed stationary phase, the arrays were placed into a desiccator overnight with an open dish containing 400 μL of n-butyldimethylchlorosilane (C4 phase; Acros Organics, NJ, USA). The arrays were then rinsed in

toluene, followed by tetrahydrofuran (Fisher Scientific, NJ, USA), a 90/10% ratio of deionized water and tetrahydrofuran, and deionized water. Each rinse lasted 10 min and was repeated twice. Finally, the array was dried under a stream of nitrogen.

Spinning devices

Two custom made spin devices were used in this work for the development and retentive capabilities of the arrays: one an adapted general spin coating device and the other a dedicated machined spinning device specific to this study (Figure. 3.3.1). The device was made from high strength, and high temperature PEEK material with a diameter of 7.6 cm and a height of 2 cm. It was equipped with FAULHABERR® 12 V, 5 mm diameter brush flat DC micromotor (MICROMO, FL, USA). By adjusting the voltage applied (0–12 V) the rotational speed could be ramped up to ~8500 rpm.

Mobile-phase velocity comparison

A 1"× 1" DW pillar array, with pillars ~200 nm in diameter, ~2 µm high, and with ~300 nm gaps, was coated with 25 nm PSO and functioned with the C4 stationary phase. Solvent flow studies were conducted to compare velocities with different spin rates and solvent compositions. A syringe pump and a capillary tube were used to deliver the solvent; each run corresponded to a discrete droplet (~5 µL) of solvent. The solvents included (i) Ethanol (Decon Laboratories, PA, USA), (ii) ACN (Fisher Scientific, NJ, USA), and (iii) Methanol (Fisher Scientific, NJ, USA) mixtures of water with these solvents were studied as well. Extensive solvent velocity studies were conducted using an iPhone 5 (Apple, CA, USA) eight-megapixel rear camera with frames collected at 30 Hz. In selected cases, video of the solvent flow was recorded using a high-speed camera system (AVT Bonito, 386 fps, Mono) with XCap Standard Version 3.8 software (Epix) in order to visualize the nuances of the solvent flow patterns. In most cases, the acquisition rate

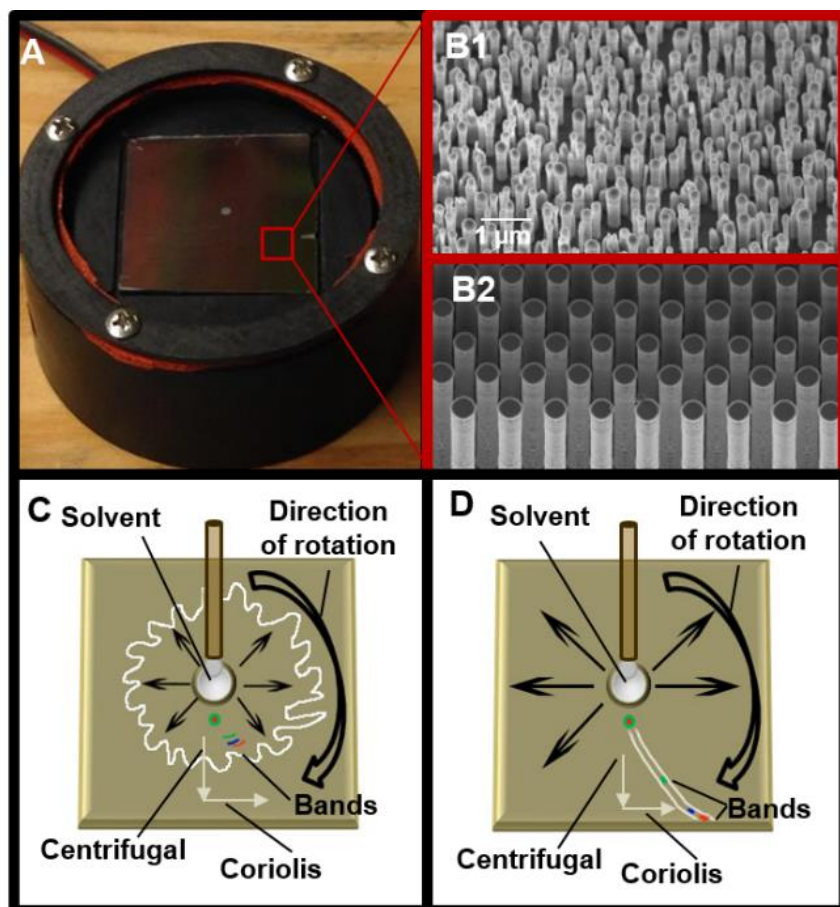


Figure 3.3.1: (A) depicts the spinner device with array, SEM images of DW (B1) and PL (B2) pillars. (C) is a model of a complete-flow development pattern and (D) is a narrow creek-like flow development pattern.

and time was 15 Hz and 0.1 ms, respectively.

Separations

Separation experiments were performed with samples composed of laser dyes consisting of Coumarin 540 (Lambda Physik, MA, USA, 1×10^{-5} M), Coumarin 440 (Sigma–Aldrich, MO, USA, 1×10^{-5} M), Sulforhodamine 640 (Exciton, OH, USA, 1×10^{-6} M) in 60% methanol 40% water. Samples were applied to the pillar arrays using a 5 μ L HPLC syringe and a CCD camera via a contact transfer spotting method.[13, 14, 28] As with the mobile phase velocity comparisons, the mobile phase was delivered using a syringe pump and a capillary tube. Mobile phases were dispensed onto spinning pillar arrays by two different manners: either as discrete droplets or by continuous flow. The syringe pump flow rate and the relative position of the capillary tube tip to the centroid of the spinning sample determined if the mobile phase was applied by discrete drops or a continuous flow. Discrete droplets were generated when lower pump flow rates were used in conjunction with the tip of the capillary tube ~ 3 mm from the spinning sample. The mobile phase was delivered by continuous flow when higher pump flow rates were used and the tip of the capillary tube < 3 mm from the spinning sample. The manner in which the mobile phase was dispensed influenced the nature of the development as either complete-flow or creek-like flow (Figure. 3.3.1C and D). Complete-flow development was observed more frequently when discrete droplets were used to deliver the mobile phase. However, most efforts produced a creek-like flow development. The four sample spots around the centroid of the array took advantage of the superhydrophobic behavior of the arrays and were performed as described previously.[13, 14] At times, the sample spots could be < 300 μ m in diameter. Separations were carried out in 60:40% or 70:30% by volume ethanol/water on C4 functionalized arrays. Fluorescence imaging of spots, before and after development, was

performed with a Nikon Eclipse E600 with Q capture software. Intensity profiles were generated from these images using Image J 1.47 V (Wayne Rashband, National Institutes of Health, USA) and public domain software A.

3.4 – Results and Discussion

Using CF during development, solvent flow was augmented to study transport, band dispersion, and separations in deterministically ordered and stochastically patterned on-chip planar pillar arrays (Figure 3.3.1). Factors controlling CF flow and performance (pillar size, rotational rate, solvent viscosity and vapor pressure, etc.) as well as the effect on analytical separation parameters were examined.

Controlling flow was important as it governs H , evident in the abbreviated form of the van Deemter equation (Eq. (3.4.1)).[13, 14] H is the common measure of band dispersion in chromatography that is optimized at smaller values. The particle diameter (or pillar dimension in our approach) is represented by d_p , while the diffusion coefficient for the solute in the mobile phase is D_M , and the independent factors specific to packing quality are γ and ω .

$$H = \frac{2\gamma D_M}{v} + \frac{(\omega)d_p^2 v}{D_M} \quad [3.4.1]$$

The existence of an optimum in linear flow velocity, v , is seen in the equation. In a prior work using similar PL pillar arrays, optimum flow rates range between approximately 0.1 and 0.7 cm/s.[29] The optimum v for the nanoscale DW arrays are greater (section 3.8 Supporting Information).

A dilemma arises in TLC since reducing d_p , to minimize the second term (resistance to mass transfer in the mobile phase) in Eq. (3.4.1), slows flow and exacerbates the first term (molecular diffusion). Thus, a means to control flow beyond simple capillary action is desirable.

While overpressure techniques have enhanced flow in TLC,[15] they require a soft seal between a confinement barrier and the separation medium, which is not easily accomplished for the diminutive on-chip systems under investigation. The CF exerted by rotation of the pillar array provides an alternative. CF progressively increases when a fluid is introduced at the center of a rotating separation platform and a resulting advancing solvent front, S_f , is produced.[30, 31]

$$CF = \int_0^{S_f} \frac{mV^2}{S_f} dS_f \quad [3.4.2]$$

The solvent mass distributed within the vicinity of the pillars up to S_f is represented by m while V^2/S_f is the angular acceleration controlled by spin rate (where V is angular velocity). The value of m is given by ρAS_f , where ρ is the solvent density and A is the porosity adjusted cross sectional area of the advancing solvent front. V is then equal to $(R/60)2\pi S_f$ where R is rotational rate (rpm). With these substitutions in Eq. (3.4.2) and subsequent integration, Eq. (3.4.3) is obtained (section 3.8).

$$CF = 0.003\rho R^2 S_f^3 \quad [3.4.3]$$

A second effect is a v -dependent Coriolis force that acts at right angles to the CF flow [30, 31]. The Coriolis force can be as large as the CF. The CF and Coriolis force vectors are observed in the positions of solute bands when separations are performed on extended 1"× 1" array platforms (Figure 3.3.1). The effect of both CF and Coriolis force are seen in sample band trajectories, whether the conditions produce a complete-flow (2D) development of the entire array (Figure 3.3.1C) or a creek-like development (Figure 3.3.1D). The creek-like development dominates when the amount of solvent and pillar height is limited and fingering creates a preferred narrow pathway for the solvent. Regardless of whether the array exhibited complete or creek-like development pattern, the slow acquisition time of the iPhone 5 resulted in snapshots

like those depicted in Figure 3.4.1. The highly textured surfaces may exhibit additional unique dynamic effects beyond observed in prior reports on smooth surfaces.[32, 33]

Patterned on-chip pillar arrays were combined with CF assisted solvent flow using spinning devices (Figure 3.3.1). Pillar array size and gaps were discussed in depth in our previous publications.[13, 14] The mobile phase velocity was varied with the spin rate and could be continuously regulated between 500 to 8500 rpm. The more the spin rate was increased, the faster the expected flow of the mobile phase. Studies were conducted investigating the velocity trends for three solvents—ethanol, ACN, and methanol—with different spin rates and solvent compositions to compare solvent flow velocities (Figure 3.4.1, Supporting Information). Selected properties of the three reverse-phase organic modifiers used in this work is provided in Supporting Information Table 3.8.2. These three solvents have very similar densities, but development times are expected to increase with higher viscosity and vapor pressure. The product of these parameters for the solvents follow the order ACN > ethanol > methanol, as do the development times (Figure 3.4.1). The most significant observation was that spinning at 3500 rpm enhances solvent velocity drastically over no spinning (Figure 3.4.1). An increase in solvent velocity as S_f increases Eq. (3.4.3) was expected but not observed. The development time was reduced with increasing spin rate for all solvent cases (Supporting Information Table 3.8.1). It is likely that the rate of evaporation increased with spin rate.

The question arises as to whether CF based flow within the pillar arrays will be large relative to capillary action and easily controllable via spin rate. The calculated CF for a representative pillar array is 15 dynes (see Supporting Information). By comparison, the force needed to induce a 0.5 cm/s flow in the same array is an order of magnitude larger than that according to the Darcy relationship. Accepting a slower flow rate and using higher spin rates

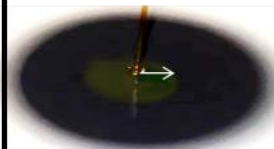
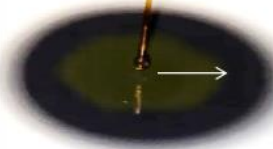
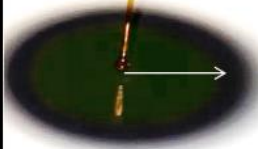
5mm		10mm		15mm	
					
Solvent	Spinner RPM	Time to develop 5mm (sec)	Time to develop 10mm (sec)	Time to develop 15mm (sec)	
Ethanol	3500	0.063	0.16	0.28	
Ethanol	Capillary force	7.7	37	112	
Acetonitrile	3500	0.076	0.12	0.18	
Acetonitrile	Capillary force	1.6	11	52	
Methanol	3500	0.096	0.19	0.49	

Figure 3.4.1: Camera snapshots of CF at various distances from centroid of spin are shown on top, and development times for various solvents under CF as well as traditional, capillary force are shown on bottom.

may make the in-pillar CF comparable to the expected resistive drag. In the case of the work presented here, however, it appears that another phenomenon is contributing to the CF enhanced separations.

Viscosity or surface tension differences at the solvent front can give rise to fingering instability on rotating smooth surfaces.[32,33] Photographs of that phenomena [32,33] look similar to the high temporal resolution images obtained of our arrays (Figure 3.4.2A). Although our platforms are textured with pillars, and hence differ, it appears that flow is occurring on top of the pillars. Co-planar flow within the pillars then accompanies the flow on top due to surface tension (Figure 3.4.2B). Microchannels with pillars lining the channel floor and with pressure-driven flow have been previously reported.[34, 35] Depending on the tilt of the pillars, secondary flows perpendicular to the floor occur. Successful separations with our vertical rigid pillars indicate that secondary flow patterns are not observed. In our system, the co-planar flow above the pillars (Figure 3.4.2B) can have a height much greater than the pillars (impacting m in Eq. (3.4.2)) and experience less drag than flow within the pillars. Hence, CF can very rapidly drive planar chromatography development.

Separations were observed in up to four quadrants of the 1"× 1" array platform; on average, approximately three co-planar creek-like flow patterns intersect sample spots and result in separations. Fluorescent images of the original spots were taken before (Figure 3.4.3A, left) and after (right) development. This was to ensure the solvent interacts with the pillar array incorporated spots, which was evident as the fluorescence of the original spot decreased drastically after development. Separations occurring during complete-flow development (Figure 3.4.3B) had bands that were less resolved and exhibited band profiles similar to prior work that solely utilized capillary action based flow [13,14]. During creek-like development (Figure 3.4.3C

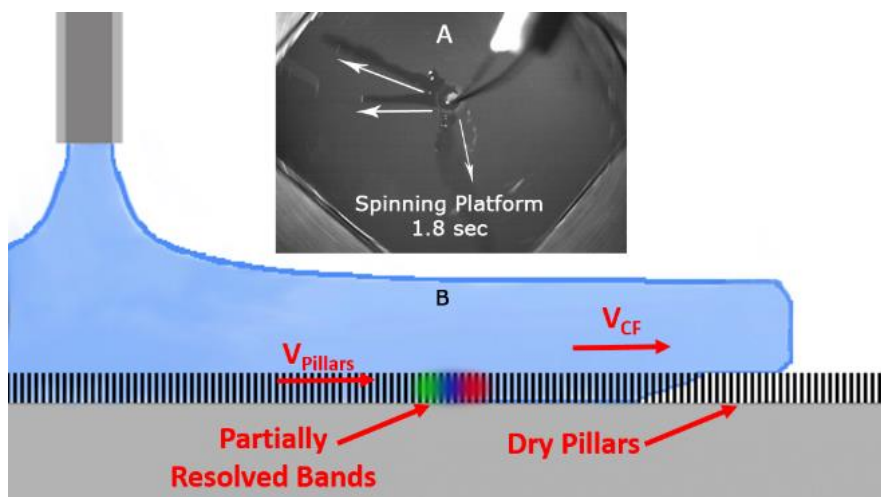


Figure 3.4.2: (A) High-resolution appearance of transient fingering 1.8 s after solvent (100% ethanol) is introduced at the centroid while rotating at 3500 rpm. (B) Cartoon depiction of the co-planar flow above and concurrently within the pillar array that drives the separation.

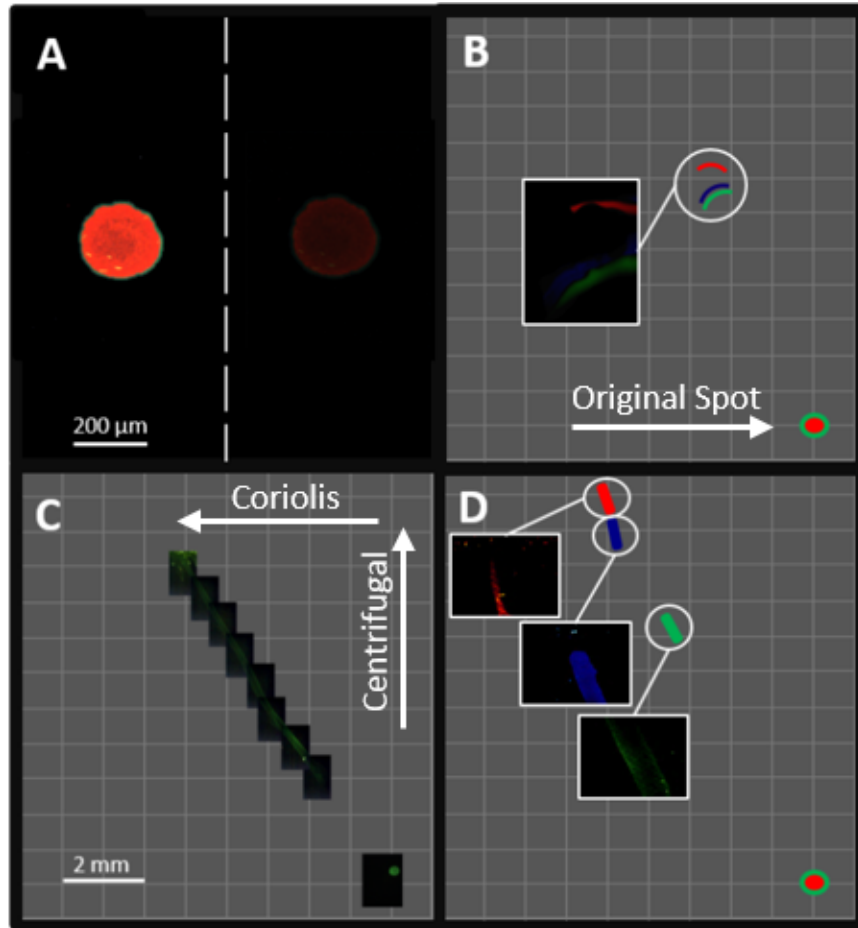


Figure 3.4.3: (A) An image of the original spot before (left) and after (right) development, (B) 3 dye component separation with complete-flow development on a DW array, (C) fluorescent images tracking the creek-like flow development, and (D) 3 dye component separation with creek-like flow development on a PL array.

and D), the solvent confined itself to a narrow finger where the direction of flow was influenced by both CF and the Coriolis force (Figure 3.4.4). While both types of development were observed, the creek-like development was more common.

Studies were conducted to determine the effects of solvent flow rate, spin rate, and solvent mixture on separation outcomes. Separations were terminated after < 15 s of development. However, the separations occurred in less time while a steady state of delivered flow counteracted evaporation during most of that period. In a comparison of solvent flow rates the flow was reduced from 2.5 $\mu\text{L}/\text{min}$ (Figure 3.4.4A) to 0.83 $\mu\text{L}/\text{min}$ (Figure 3.4.4B). The development distance reduced and the R_f value was not affected and the plate height increased, presumably due to less development distance (Supporting Information Table 3.8.3). In reducing the spin rate from 3500 (Figure 3.4.4A) to 1500 rpm (Figure 3.4.4C) the plate height increased. The development distance did not decrease, presumably due to the ease with which the co-planar flow occurs above the pillars. The retention decreased (R_f increase) due to the use of a different array, since the stationary phase creation chemistry has some variability and influences phase ratio. With an increase in the mobile-phase strength from 60:40 ethanol/water (Figure 3.4.4A) to 70:30 ethanol/water (Figure 3.4.4D) retention was decreased (R_f increase) and the efficiency degraded (Supporting Information Table 3.8.3).

3.5 – Conclusions

In all development cases, the test dyes were successfully separated quickly with plate heights ranging from 1.5 to 0.21 μm . In the current chip design, reproducibility is limited by the random nature of creek-like fingers that drive development and separation. The nature of the drying process after development also plays a part in the appearance of the bands.[14] Nevertheless, the efficiency and speed of separation obtained are promising and attributable to

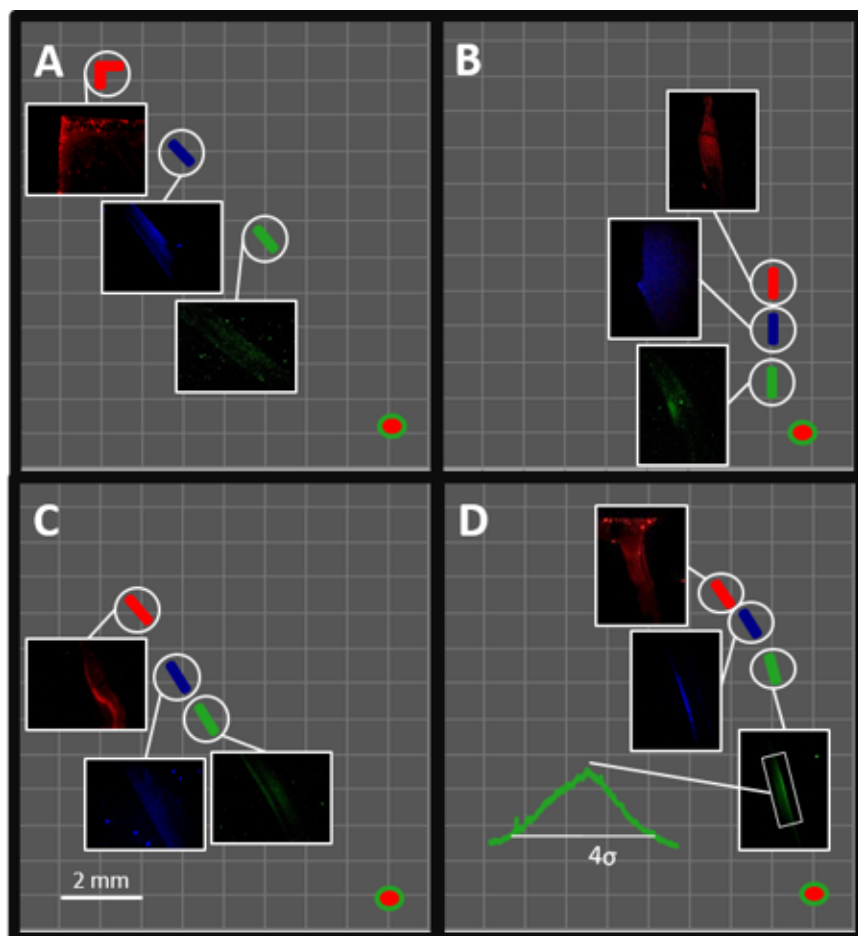


Figure 3.4.4: Depicts four 3 dye component separations with creek-like flow development on DW arrays at varying conditions. In (A) separation carried out with solvent flow rate of 2.5 $\mu\text{L}/\text{min}$, spin rate 3500 rpm, and a 60:40 ethanol/water solvent mixture. In (B–D) one of the standard conditions change. In (B) the solvent flowrate is reduced to 0.83 $\mu\text{L}/\text{min}$, (C) the spin rate is reduced to 1500 rpm, and (D) the solvent mixture is 70:30 ethanol/water.

the uniqueness of the observed co-planar CF driven flow. This rapid co-planar flow reverses the evolving slow development of conventional capillary action driven flow and the concomitant molecular diffusion related spot broadening. Future work will include photolithographic production of channels and subsequent patterned DW processing to create pillars within the channels. It is expected that pillars within the narrow (≤ 1 mm) spoke-like channels will reproducibly confine the co-planar flow within programmable regions on the chip, which will make co-planar CF flow to drive planar separations more practical and reliable.

3.6 – Acknowledgments

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3.7 – References

- [1] Poole, C. F., *J. Chromatogr. A* 2003, 1000, 963–984.
- [2] Poole, C. F., *J. Chromatogr. A* 1999, 856, 399–427.
- [3] M riziq, K. S., Guiochon, G., *J. Chromatogr. A* 2008, 1187,180–187.
- [4] Rabel, F., Sherma, J., *J. Liq. Chromatogr. Relat. Technol.*2016, 39, 385–393.
- [5] H`abe, T. T., Morlock, G. E., *Chromatographia* 2016, 79,797–810.
- [6] Poole, S. K., Poole, C. F., *J. Chromatogr. A* 2011, 1218,2648–2660.
- [7] Patel, R. B., Gopani, M. C., Patel, M. R., *Chromatographia* 2013, 76, 1225–1231.
- [8] Hauck, H. E., Schulz, M., *J. Chromatogr. Sci.* 2002, 40,550–552.
- [9] Jim, S. R., Foroughi-Abari, A., Krause, K. M., Li, P., Kupsta, M., Taschuk, M.T.,Cadien, K.C., Brett, M.J., *J. Chromatogr. A* 2013, 1299, 118–125.
- [10] Jim, S. R., Oko, A. J., Taschuk, M. T., Brett, M. J., *J. Chromatogr. A* 2011, 1218, 7203–7210.
- [11] Song, J., Jensen, D. S., Hutchison, D. N., Turner, B., Wood, T., Dadson, A., Vail, M. A., Linford, M. R., Vanfleet, R. R., Davis, R. C., *Adv. Funct. Mater.* 2011, 21,1132–1139.
- [12] Clark, J. E., Olesik, S. V., *Anal. Chem.* 2009, 81,4121–4129.
- [13] Kirchner, T. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., *Anal. Chem.* 2013, 85, 11802–11808.
- [14] Kirchner, T. B., Strickhouser, R. B., Hatab, N. A., Charlton, J. J., Kravchenko, II, Lavrik, N. V., Sepaniak, M. J., *Analyst* 2015, 140, 3347–3351.
- [15] Dzido, T. H., Plocharz, P. W., Slazak, P., *Anal. Chem.* 2006,78, 4713–4721.
- [16] Nyiredy, S., *J. Chromatogr. A* 2003, 1000, 985–999.
- [17] Kalasz, H., *J. Chromatogr. Sci.* 2015, 53, 436–442.

- [18] Nurok, D., Koers, J. M., Novotny, A. L., Carmichael, M. A., Kosiba, J. J., Santini, R. E., Hawkins, G. L., Replogle, R. W., *Anal. Chem.* 2004, 76, 1690–1695.
- [19] Moricz, A. M., Kalasz, H., *JPC J. Planar Chromatogr. Mod. TLC* 2010, 23, 415–419.
- [20] Hostettmann, K., Hostettmannkaldas, M., Sticher, O., *J. Chromatogr.* 1980, 202, 154–156.
- [21] Stahl, E., Muller, J., *Chromatographia* 1982, 15, 493–497.
- [22] Singh, P., Rawat, B., Rawat, B. S., Pant, G. J. N., Rawat, M. S. M., Joshi, G. C., *J. Indian Chem. Soc.* 2014, 91, 1555–1559.
- [23] Agrawal, V., Desai, S., *J. Pharmacogn. Phytochem.* 2015, 3, 145–149.
- [24] Bernard-Savary, P., Poole, C. F., *J. Chromatogr. A* 2015, 1421, 184–202.
- [25] Agapov, R. L., Srijanto, B., Fowler, C., Briggs, D., Lavrik, N. V., Sepaniak, M. J., *Nanotechnology* 2013, 24, 505302–11.
- [26] Charlton, J. J., Lavrik, N., Bradshaw, J. A., Sepaniak, M. J., *ACS Appl. Mater. Interfaces* 2014, 6, 17894–17901.
- [27] Lincoln, D. R., Lavrik, N. V., Kravchenko, I. I., Sepaniak, M. J., *Anal. Chem.* 2016, 88, 8741–8748.
- [28] Wallace, R. A., Charlton, J. J., Kirchner, T. B., Lavrik, N. V., Datskos, P. G., Sepaniak, M. J., *Anal. Chem.* 2014, 86, 11819–11825.
- [29] Crane, N. A., Lavrik, N. V., Sepaniak, M. J., *Analyst* 2016, 141, 1239–1245.
- [30] Nolte, D. D., *Rev. Sci. Instrum.* 2009, 80, 101101–22.
- [31] Brenner, T., Glatzel, T., Zengerle, R., Ducree, J., *Lab Chip* 2005, 5, 146–150.
- [32] Troian, S. M., Wu, X. L., Safran, S. A., *Phys. Rev. Lett.* 1989, 62, 1496–1499.
- [33] Alvarez-Lacalle, E., Ortín, J., Casademunt, J., *Phys. Fluids* 2004, 16, 908–924.
- [34] Masoud, H., Alexeev, A., *Chem. Commun.* 2011, 47, 472–474.

[35] Branscomb, J., Alexeev, A., *Soft Matter* 2010, 6,4066–4069.

3.8 – Supporting Information

Efficiency and other separation parameters

Referencing text Equation 3.4.1, and using typical values for λ and ω of 0.5 and 0.02,[1-3] respectively, Equation 3.8.1 is created for the optimum flow velocity, v_{opt} .

$$v_{opt} = \left[\frac{B - term}{C_m - term} \right]^{1/2} = \left[\frac{2(0.5)D_m^2}{(0.2)d_p^2} \right]^{1/2} \quad [3.8.1]$$

Letting $D_m = 5 \times 10^{-6} \text{ cm}^2/\text{s}$ and $d_p = 2.5 \times 10^{-5} \text{ cm}$ the value for v_{opt} is 2 cm/s. Considering the ethanol development times in text Figure 3.4.1, the velocities at 3500 RPM for flow 0-5mm, 5-10 mm, and 10-15 mm velocities are approximately 8, 5, and 4 cm/sec, respectively. For ethanol under simple capillary action flow, the values are approximately 0.07, 0.02, and 0.01 cm/sec. Clearly, molecular diffusion dominates the capillary action case. Conversely, the CF-driven case is a bit too fast and resistance to mass transfer is significant. However, it may be possible to lower the spinning rate to compensate by reducing flow rate (see Supplemental Information Table 3.8.1). The experimental data associated with Figure 3.4.4 yields the plate heights (H in μm) shown below. These plate heights are computed as

$$H = \frac{(W_f - W_i)^2}{16d} \quad [3.8.2]$$

where W_f and W_i are the final and initial band widths and d is the distance the band traveled during development. Intensity profiles were generated using unprocessed images prior to any adjustments in brightness and contrast (for better visualization in text Figure 3.4.4) and used to measured band widths. The retardation factor R_f is d/S_f with S_f assumed to be the position of the unretained Sulforhodamine dye. The duplicate run for 4D in Table 3.8.3 demonstrate a low level of reproducibility which is limited by the random (width, length, depth, timing) nature of creek-

like fingers that drive development and separation. Variations in the co-planar (above and within pillars) creek-like flow pattern, dimensions, and whether more than one creek develops at a given timeframe influences separation performance. The nature of the drying process after development also plays a part in the appearance of the bands. These variables influence separation efficiency, distance traveled, etc. However, in all development cases, the test dyes were successfully separated in a very short time. Future work will include photolithographic production of channels and subsequent DW processing to reproducibly confine the creek-like flow.

Table 3.8.1: Development rates for different spin rates and solvents

Solvent	Spinner RPM	Time to Develop 5 mm (sec)	Time to Develop 10 mm (sec)	Time to Develop 15 mm (sec)
Ethanol	1000	0.23	0.33	0.50
Ethanol	2500	0.12	0.15	0.30
Ethanol	3500	0.063	0.16	0.28
Ethanol	0	7.7	37	112
70/30 EtOH/H ₂ O	1000	0.80	1.5	2.4
70/30 EtOH/H ₂ O	2500	0.23	0.26	0.39
70/30 EtOH/H ₂ O	3500	0.09	0.22	0.33
Acetonitrile	1000	0.23	0.26	0.39
Acetonitrile	2500	0.14	0.17	0.26
Acetonitrile	3500	0.076	0.12	0.18
Acetonitrile	0	1.6	11	52
70/30 ACN/H ₂ O	1000	0.54	0.90	1.80
70/30 ACN/H ₂ O	2500	0.16	0.21	0.40
70/30 ACN/H ₂ O	3500	0.10	0.18	0.30
Methanol	1000	0.22	1.06	1.94
Methanol	2500	0.26	0.24	0.60
Methanol	3500	0.096	0.19	0.49

Table 3.8.2: Properties of the reversed phase organic modifiers

Solvent	Density	Viscosity	Vapor Pressure	Polarity Index
Acetonitrile	0.79	0.34	73	5.8
Ethanol	0.79	1.08	45	4.3
Methanol	0.79	0.54	160	5.1

Analytical model

Centrifugal Force driven flow in a creek-like flow pattern:

S_f – radial position, CF driving force

$$CF = \frac{mV^2}{S_f}$$

$$m = \rho AS_f \text{ and } V = \left(\frac{R}{6}\right) 2\pi S_f$$

m – solvent mass, ρ – solvent density, A – channel cross section (adjusted for porosity),

V – rotational velocity, R – rpm

$$CF = 0.003\rho AR^2 S_f^3 \quad [3.8.3]$$

CF for a photolithographic array with pillar height 20 μm , channel (creek width) 500 μm , $S_f = 2$ cm, porosity factor 50% yields $A = 5.0 \times 10^{-5} \text{ cm}^2$.

For a solvent with a density of 1.0 g/cm^3 (assume water) and $R = 3,500$ RPM the calculated CF using the equation above is 15 dynes. At just over 10,000 RPM this would be ~ 120 dynes.

Table 3.8.3: Efficiency and retention (dimensions in μm)

Figure	C540 Band Width	Orig. Spot Width	R _f	Distance Traveled	Plate Height	Chip
4A	380	240	0.48	5830	0.21	Z
4B	400	290	0.45	2060	0.37	Y
4C	540	320	0.68	6730	0.45	Y
4D1	660	255	0.63	6870	1.5	X
4D2	600	250	0.59	5220	1.5	X

Resistive force:

Using the Darcy Equation and experimental flow resistance parameter (Φ) for a typical photolithographic pillar array of 48,[3] we have the following.

$$\Delta P = \frac{force}{A} = \frac{v\eta S_f \Phi}{d_p^2} \quad [3.8.4]$$

Assume the following parameters:

d_p - pillar diameter (2 μm), v – flow velocity (0.5 cm/s), A is $5 \times 10^{-5} \text{ cm}^2$ as above,

η - viscosity 0.01 g.(s cm)

The force computes to be 600 dynes (much larger than CF within pillar array, see above). This suggests an alternate basis for rapid CF-based flow (see manuscript text and Figure 3.4.2).

References

- [1] Kirchner, T. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., Anal. Chem. 2013, 85, 11802-11808.
- [2] Kirchner, T. B., Strickhouser, R. B., Hatab, N. A., Charlton, J. J., Kravchenko, II, Lavrik, N. V., Sepaniak, M. J., Analyst 2015, 140, 3347-3351.
- [3] Lavrik, N. V., Taylor, L. C., Sepaniak, M. J., Lab on a Chip 2010, 10, 1086-1094.

Chapter 4

Efforts in Increasing Control of Solvent Flow in Centrifugal-Driven, Reduced-Dimension, Planar Chromatography Systems

4.1 – Abstract

In an effort to overcome problems with efficiency due to diminishing flow rate of capillary driven planar chromatography, our previous work applied centrifugal force to reduce band dispersion in nanoscale TLC features.[1] This work demonstrated the first analytical separation on pillar arrays using centrifugal force to increase solvent flow velocities. Even though separations were achieved, success was limited by the lack of reproducibility. To increase control over the creek-like fingers that drive the development and separation, we have explored the use photolithography to create channels and nanoscribe structures to contain and direct the development of centrifugal driven, reduced dimension, planar chromatography platforms.

4.2 – Experimental

Fabrication of pillar arrays

Stochastic pillar arrays were created (as described in our previous work) on p-type silicon wafers with 100 nm of thermally grown SiO₂ using the lithography free metal dewetting method.[1, 2] A dual beam electron gun evaporation chamber was used to vapor deposit a thin film of platinum (8-10 nm; Thermonics Laboratory, VE-240). The deposition rate and thickness of the platinum was monitored with a quartz crystal. The thin platinum film was then rapidly heated to 900°C to create platinum islands in a rapid thermal processor cold wall furnace (Easy Tube 3000, First Nano) using a 10:1 mixture of argon and hydrogen at 735 torr. The platinum islands functioned as a mask for anisotropic reactive ion etching (Oxford Plasma Lab, Oxford Instruments) of the substrate, as characterized and described in earlier work.[3, 4]

Fabrication of etched channels

Channels were designed using CAD software and fabricated using photolithography on p-type silicon wafers. A hard mask of 20 nm of alumina was deposited on the silicon wafer using

atomic layer deposition (FlexAL Atomic Layer Deposition System, Oxford Instruments). Photoresist (955CM 2.1, Microchem Corp.) was spin coated onto the wafer followed by exposure to UV light through the CAD designed mask on a MA6/BA6 mask aligner (Süss MicroTech SE.). After development (CD-26 Developer, Microchem Corp.) a 3-minute sputter etching of the alumina mask, followed by Bosch etching, was carried out on a RIE/ICP Etcher (Plasmalab 100, Oxford Instruments) to reach a channel depth of 50-100 μm . The remaining resist was stripped in a hot NMP (Remover 1165, Microchem Corp.) bath for 30 minutes followed by a max strip treatment in a TePla (IoN Wave 10, PVA TePla America), then baked clean in a rapid thermal processor (RTP, First Nano) with hydrogen and argon at 800°C for 15 minutes. The wafer was oxidized at 1100°C in a rapid thermal processor cold wall furnace (Easy Tube 3000, First Nano). This was followed by the creation of stochastic pillar arrays as described above. Figure 4.2.1 show the various channel designs created using this process. A common characteristic of all designs is a central feature where solvent (mobile phase) can be applied and a radial flow realized.

Fabrication of PDMS channels

Channels were also created on stochastic pillar array substrates by bonding molded PDMS (polydimethylsiloxane, Dow Corning Corp.). The PDMS was mixed 10:1 base to curing agent, degassed in a desiccator for 20 minutes, poured onto a silicon mold, and cured in an oven at 70°C for 1 hour. Once cooled, a thin layer (50 nm) of alumina was deposited via atomic layer deposition (FlexAL Atomic Layer Deposition System, Oxford Instruments) on the PDMS. The molded PDMS was bonded to the substrate using a plasma cleaner (Harrick Plasma) or by glue. Using the plasma cleaner, the molded PDMS and the substrate were placed on a glass slide and exposed to oxygen plasma for 20 seconds. The PDMS was then placed on top of the substrate to

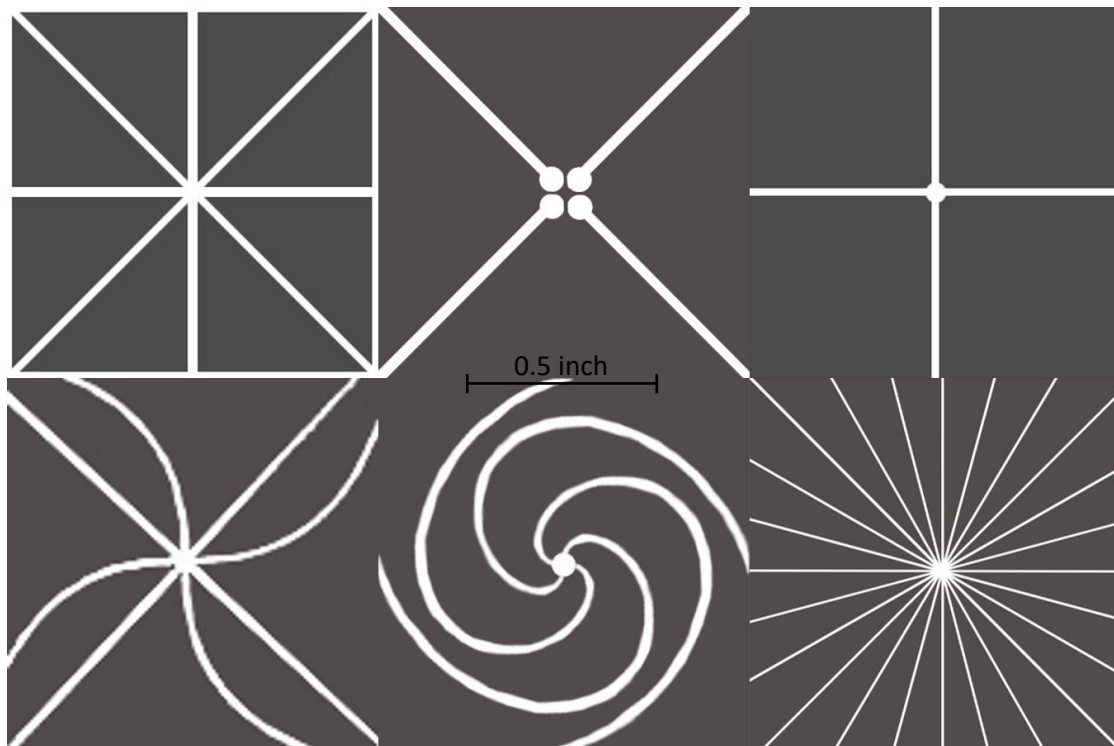


Figure 4.2.1: Channel designs.

form channels. Bonding using glue was carried out after deposition of alumina. The molded PDMS was painted with a thin coat of uncured PDMS or stamped onto a substrate with a layer of spun coated PDMS. The molded PDMS was placed atop the substrate and placed in an oven at 70°C for 1 hour to cure and bond.

Fabrication of SU-8 channels

Photolithography was used to create channels on top of substrates patterned with stochastic pillar arrays. The substrates were baked at 250°C for 30 minutes prior to being spun coated with SU-8 photoresist (Microchem Corp.) and baked. Substrates were exposed to UV light through the CAD designed mask on a MA6/BA6 mask aligner (Süss MicroTech SE.) followed by a post exposure bake and development.

Fabrication of nanoscribe structures

Nanoscribe structures were designed, saved in a STL format, and converted to GWL scripts using DeScribe (Nanoscribe GmbH). A 1" × 1" pillar array substrate was loaded onto the Nanoscribe Pro GT (Nanoscribe GmbH) holder and resist (IP-S, Nanoscribe GmbH) was applied. The holder was loaded into the Nanoscribe and the designed structure was printed using a 800-nm femtosecond laser excitation source focused with a 25x microscope objective. After exposure, the substrate was developed for 20 minutes in 1-methyloxy-2-propanol acetate (SU-8 developer, Microchem Corp.), rinsed with 2-propanol (J.T. Baker), and dried under a stream of nitrogen. Figure 4.2.2 show the various nanoscribe structure designs.

Deposition of porous silicon oxide

A layer of porous silicon dioxide (PSO) was deposited onto the pillared substrates using low temperature plasma enhanced chemical vapor deposition. A 25 nm layer of PSO was deposited using a PECVD System 100 Plasma Deposition Tool (Oxford Instruments). During

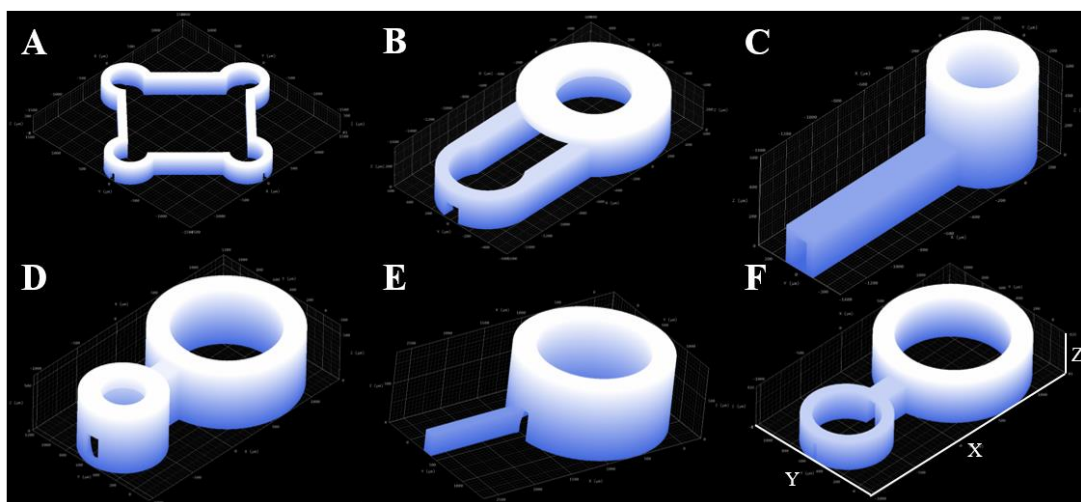


Figure 4.2.2: (A-F) illustrate the various nanoscribe structure designs. The structures range in size X: 2000-3000 μm , Y: 1200-2000 μm , and Z: 300-900 μm . The design illustrated in E has been most successful at generating thin creek-like fingers of solvent.

deposition, the chamber pressure and substrate temperature was 600 mTorr and 27°C.

Functionalization

To create reverse stationary phases, a gas-phase functionalization was carried out in which the arrays were placed into a desiccator with an open dish of 400 μ L of n-butyldimethylchlorosilane (C4 phases; Acros Organics) for 24 hours. This reagent reacts with silanol groups on the PSO. The arrays were removed from the desiccator and rinsed for 10 minutes in toluene (Fisher Scientific), tetrahydrofuran (Fisher Scientific), 90:10 ratio of deionized water and tetrahydrofuran, and deionized water. Each rinse was repeated twice. Once rinsed, the arrays were dried under a stream of nitrogen.

Separation and solvent flow experiments

Separation experiments were carried out using sample mixtures composed of laser dyes. Application of sample spots (spotting) was carried out using a 5 μ L HPLC syringe and a CCD camera via the contact transfer method as described previously in Chapters 1 and 3, as well as previous work.[2, 5, 6] Sample spots were applied within the first 3 millimeters of the channels or the opening of the nanoscribe central feature. Separation and solvent flow experiments were carried out on functionalized substrates with a 70:30 ethanol and water solvent mixture applied using a syringe pump as described in previously Chapter 3.

Imaging

A scanning electron microscope (Carl Zeiss, Merlin) was used to evaluate the pillars, channels, and nanoscribe features. Imaging of the solvent flow patterns on the substrates was recorded using a high-speed camera (AVT Bonito, 386 fps, Mono) with XCap Standard Version 3.8 software (Epix). Fluorescent imaging of chemical separations was performed using a Nikon Eclipse E600 microscope with Q-Capture software.

4.3 – Results and Discussion

Etched channels

Channels were designed to increase reproducibility by confining solvent flow during development. Straight channels did confine flow; however, the solvent would travel down the sidewall of the channel, pushed there by the Coriolis force before flowing down the bed of the channel enough to interact with the original spot. Narrower channels were designed to compensate for the Coriolis force driving flow to the channel wall, but sample application then became impossible as the channels were too narrow to spot in. Curved channels were designed to take advantage to the Coriolis force; however, the solvent flow took on a parabolic shape staying close to both sidewalls and not traveling down the channel far enough to for separation to occur. Spin rates were changed but did not have an appreciable effect on the solvent flow pattern. An increase or decrease in the solvent delivery rate or volume resulted in flooding of the channel or solvent evaporation without separation. In these designs, the channels were etched into the wafer followed by metal dewetting to create the stochastic pillars. With this channel fabrication method the sidewalls also had pillars, making them better at wicking the solvent than the bed of the channel. Therefore, anytime the solvent stayed confined to the channel, the flow would travel along the walls without sufficiently wetting or flowing along the bottom of the channel.

PDMS channels

To avoid the pillared sidewalls, channels were created by stamping molded PDMS onto substrates pattern with stochastic pillar arrays. However, this configuration resulted in incomplete bonding of the PDMS to the substrate by both plasma bonding and heated bonding. Incomplete bonding allowed the solvent to flow under the PDMS and did not keep the analyte confined to the channels (Figure 4.3.1). As the laser dye analytes migrated with the solvent flow

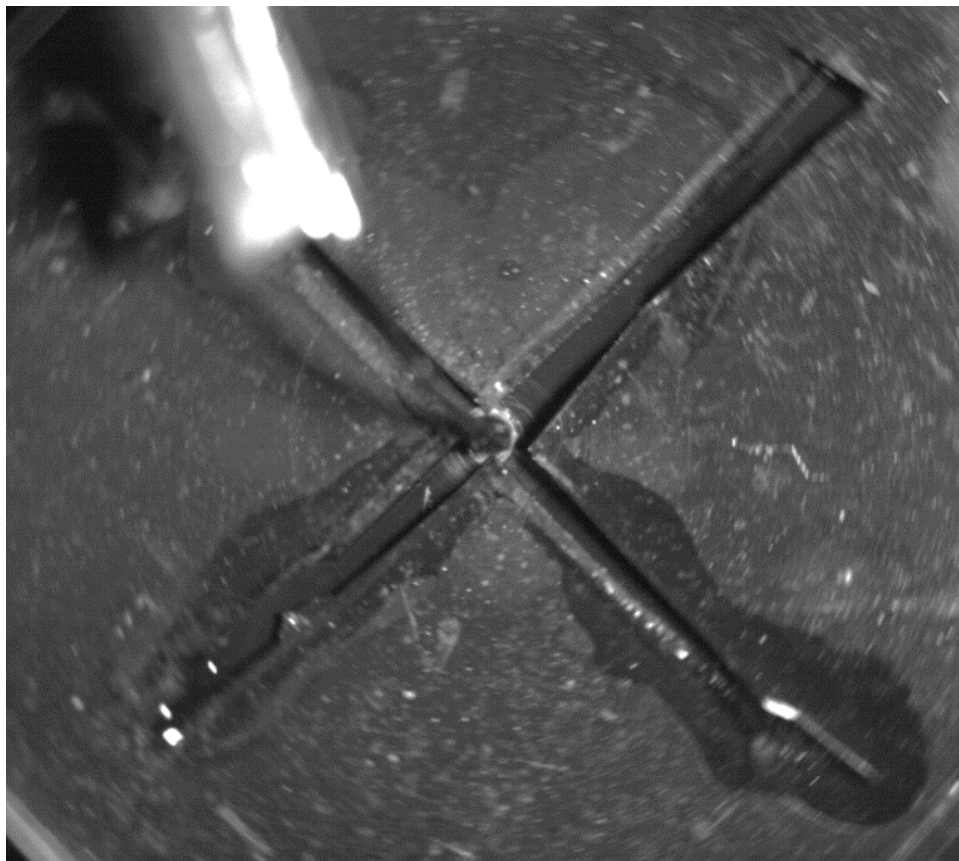


Figure 4.3.1: Illustration of the solvent flow on PDMS channeled substrate.

under the PDMS and down the channels, they were absorbed into the PDMS. This absorption resulted in permanent contamination of the substrate as the analytes were not removed from under the PDMS when washed. Therefore, the substrates were no longer reusable. A layer of alumina was deposited by ALD onto the bottom and sidewalls of the PDMS with the intention of sealing the it to eliminate analyte absorption. However, it was unsuccessful and the analyte was still absorbed by the PDMS.

SU-8 channels

To overcome the problems with the PDMS not completely bonding to the substrate patterned with stochastic pillars, photolithography was used to create channels that were made of SU-8 photoresist. This solved the bonding problem as the SU-8 did completely bond to the substrate. However, after extensive experimentation we were unable to find processing parameters that resulted in open channels due to the thickness of the polymer. The channels constantly had reflow and shape distortion issues resulting in obstructed channels, seen in Figure 4.3.2. Additionally, any attempt to visualize the sample in the channels was obscured by the highly fluorescent SU-8 polymer. As a result, the use of fabricated narrow channels to increase reproducibility of separations was abandoned.

Nanoscribe structures

Since all efforts of confine the flow were unsuccessful, nanoscribe structures were designed to direct solvent flow. Preliminary experiments, discussed in Chapter 3, indicated that thin creek-like fingers of solvent propagating from the center straight to the edge or curving to the corner driven by the Coriolis force (Figure 4.3.3) yielded the most efficient separations. A multitude of designs were fabricated (Figure 4.3.4). Even with the central feature, producing the desired flow pattern was difficult as the solvent tended to completely encircle the central feature

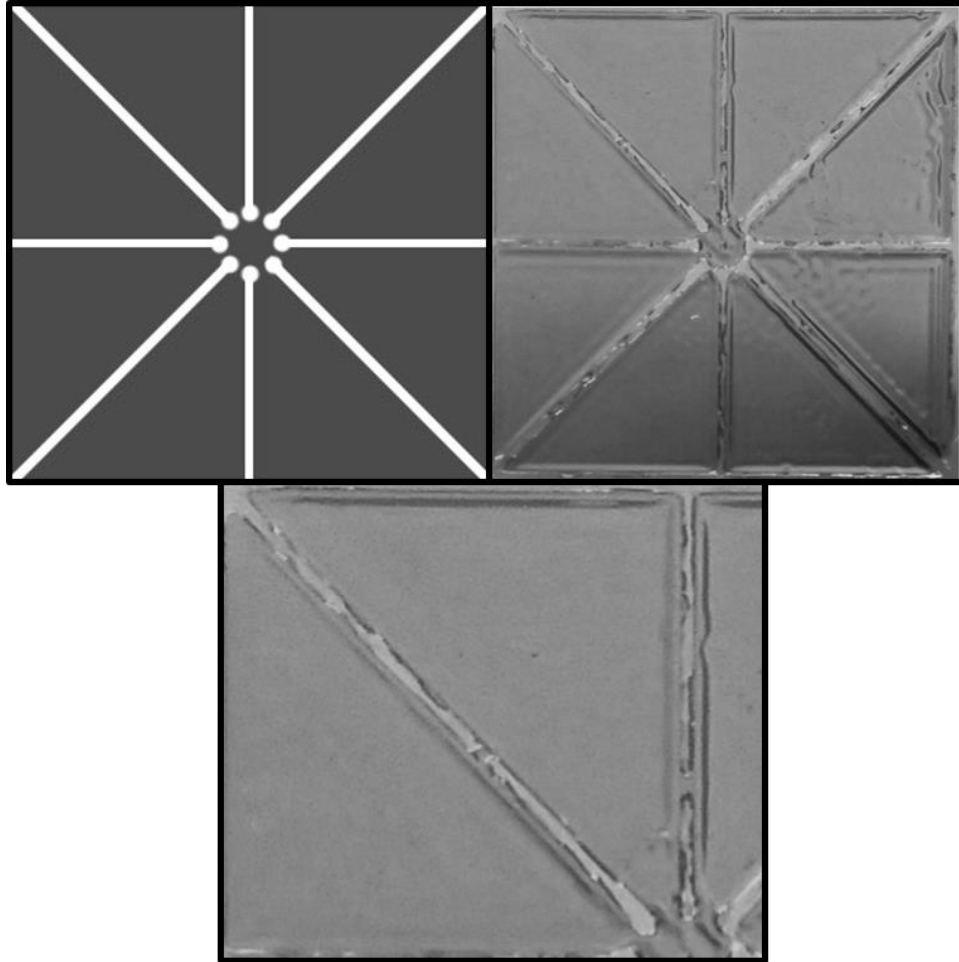


Figure 4.3.2: SU-8 channel design (left) and SU-8 fabricated channels (right and bottom).

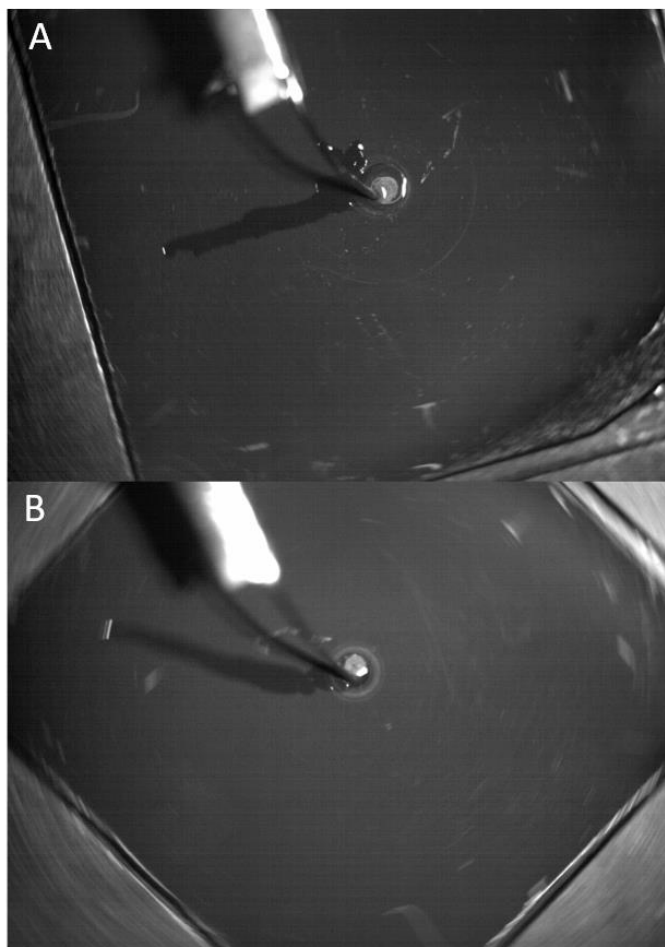


Figure 4.3.3: Depictions of the desired solvent flow pattern for separations: (A) is the solvent flow moving straight out from the center, and (B) is the solvent flow directed to the corner by the Coriolis force.

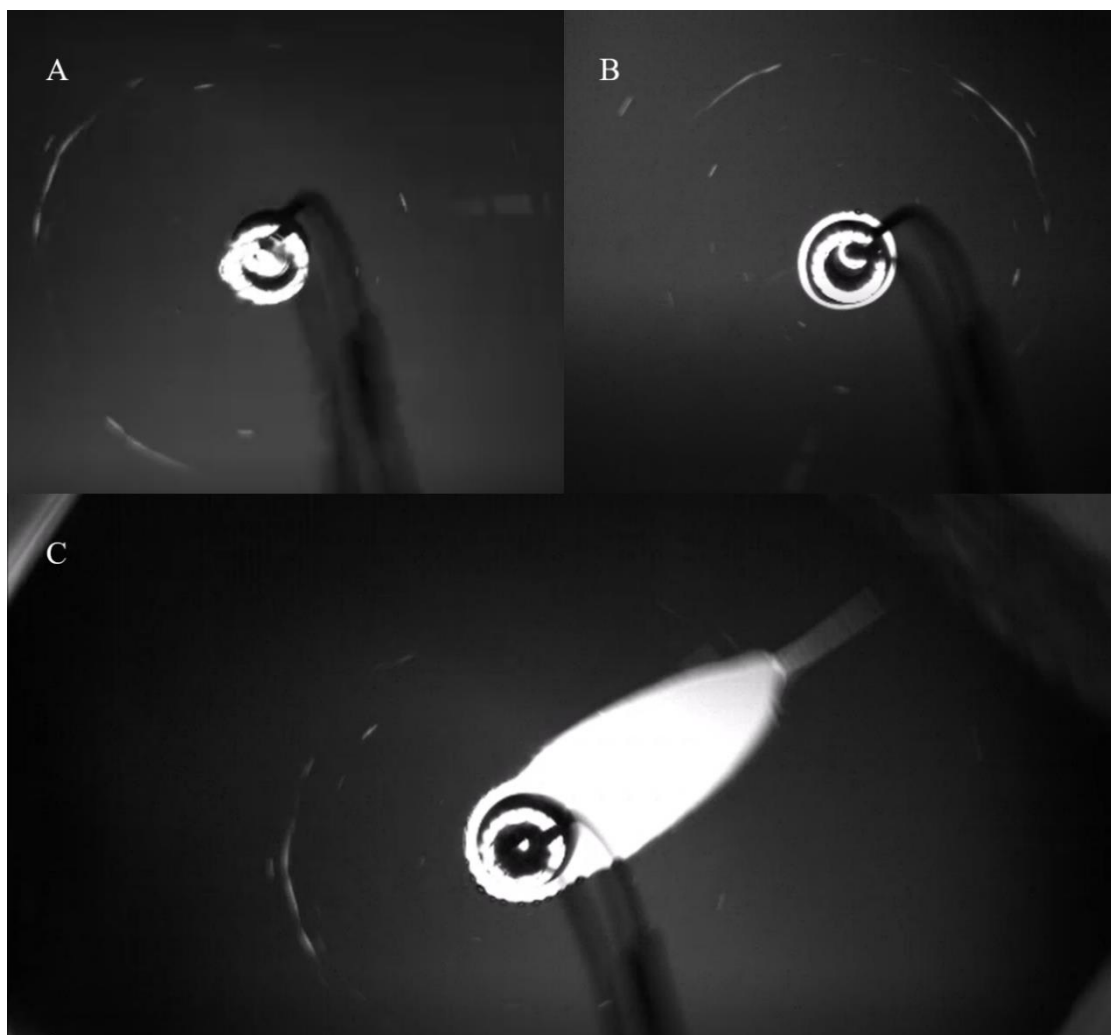


Figure 4.3.4: Illustrates the nanoscribe printed central feature before solvent applied (A), after the solvent has encircled the feature (B), and the resulting solvent flow pattern (C).

(Figure 4.3.4 B) before being directed outward by centrifugal force. This also resulted in a creek the diameter of the central feature that was too wide for efficient separation (Figure 4.3.4 C). The most successful structure is depicted in Figure 4.2.2 E. With this feature design the solvent traveled down the protrusion of the central feature long enough for the centrifugal force to overcome the solvent's affinity to the nanoscribe polymer as seen in Figure 4.3.4. As a result, the solvent did not encircle the central feature and instead traveled to edge of the substrate in the desired narrow creek-like flow pattern.

4.4 – Summary

All attempts to confine flow to channels in an effort to increase reproducibility were unsuccessful. Promising results have been obtained through the uses of nanoscribe features to augment the natural solvent flow seen in previous work (Chapter 3), which resulted in successful separations. Controlling the volume and flow rate, along with nanoscribe features to direct but not confine solvent flow, have produced an increase in the development of narrow creek-like flow. However, further experimentation is needed to fine tune the exact parameters to reproducibly obtain creek-like flow for separations. Due to time constraints, my focus turned to particle separations using nanoscribe printed meshes as described in Chapter 5. Research into this Chapter 4 project may continue at Oak Ridge National Laboratory's Center for Nanophase Materials Sciences.



Figure 4.3.5: Illustrates the most promising central feature (top) and the narrow creek-like solvent flow pattern the feature produces (bottom).

4.5 – References

- [1] Strickhouser, R. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., *ELECTROPHORESIS* 2018, 39, 438-444.
- [2] Kirchner, T. B., Strickhouser, R. B., Hatab, N. A., Charlton, J. J., Kravchenko, II, Lavrik, N. V., Sepaniak, M. J., *Analyst* 2015, 140, 3347-3351.
- [3] Agapov, R. L., Srijanto, B., Fowler, C., Briggs, D., Lavrik, N. V., Sepaniak, M. J., *Nanotechnology* 2013, 24, 505302.
- [4] Charlton, J. J., Lavrik, N., Bradshaw, J. A., Sepaniak, M. J., *ACS Applied Materials & Interfaces* 2014, 6, 17894-17901.
- [5] Kirchner, T. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., *Anal. Chem.* 2013, 85, 11802-11808.
- [6] Wallace, R. A., Charlton, J. J., Kirchner, T. B., Lavrik, N. V., Datskos, P. G., Sepaniak, M. J., *Anal. Chem.* 2014, 86, 11819-11825.

Chapter 5

Nanoscribe Printed Mesh Filters for Particle Separations

5.1 – Abstract

The aqueous sorting and separation of micro- and nanoscale particles is a useful technique in chemical, medical, and biological fields. The work herein describes the fabrication and evaluation of two microfluidic devices for the separation and sorting of high value, low volume micro- and nanoparticles. The separation systems in this work were fabricated in a serial multi-method process. First, photolithography combined with reactive ion etching was implemented to create channels in silicon substrates. Second, a Nanoscribe was employed using two-photon polymerization based 3D laser writing to effectively print tunable meshes, creating physical barriers that selectively separate particles based on size. In the second device, mesh filters top a micron sized hole. The two devices fabricated are driven by different mass transfer methods: one by capillary flow in channels, referred to as the capillary flow device (CFD), and the other by diffusion, referred to as the diffusion device (DD).

5.2 – Introduction

Separations of both biological and synthetic, micro- and nanoscale particles have a variety of applications in biology, chemistry, medicine, as well as industry. Hydrodynamic chromatography, field flow fractionation, and electrophoresis are a few of the traditional techniques that have been employed for large scale particle separations.[1-5] To achieve these separations in the modern era, microfluidic lab-on-a-chip (LOC) devices have been employed. The small size of LOC platforms makes them ideal for small sample volumes, rapid analysis, and portability.[4, 6, 7] Typically, microfluidic devices employ either active or passive techniques for particle separation or sorting. Active separation methods use external influences such as acoustic, electric, magnetic and optical fields. Alternatively, passive methods use the interaction between

the particles, the flow field, and the microchannel structures.[5] Sorting methods depend on physical properties, structure, morphology, and/or chemical characteristics to separate particles.

Microfluidic devices that employ active methods of separation are typically used for microparticles and can experience difficulties when they are used for nanoscale particle separation. The effect of the sorting forces is often diminished with size. However, this diminishing effect can be overcome by increasing the frequency and magnitude of the sorting force.[3] Passive methods of sorting can be beneficial as they do not require extra steps or equipment such as labeling or field generators. Additionally, passive methods of separation mitigate damage to the sample due to energy input. Specifically, mesh filters with micro- and nanoscale pores are useful in sorting and separating particles of different sizes without expensive setups or equipment.

Various techniques have been employed for the fabrication of porous mesh filters. Molding procedures have been developed using polymer substrates, but these devices have limited geometry and resolution.[8, 9] Other fabrication methods such as directed or self-assembly photolithography and salt-leaching have higher resolution, but do not allow for precise control of pore size and shape, especially for separation of nanoscale particles. [8, 10] The two-photon polymerization (2PP) by femtosecond laser pulses, used in this work, is a powerful method for fabricating high resolution 3D micro- and nanostructures for microfluidic LOC technologies. 2PP allows for the highly controlled printing of 3D structures with simple processing, sub-micron resolution, and easy integration into microfluidic devices. This technique uses focused near-infrared femtosecond laser pulses to polymerize a photosensitive resist. The two-photon absorption process allows for selective polymerization of the resist in a nanoscale focal volume (voxel).

While most microfluidic devices are aimed at continuous flow particle separation, the work described herein is a passive sorting method using the 2PP fabrication of microstructures aimed at separating low volume, high value samples with particle sizes ranging from nano- to micrometers. The purpose of this work was to develop microfluidic devices using photolithography and 3D micro printing, in which nanoparticles could be separated in sample volumes less than 100 nanoliters. To this end, two devices were fabricated the CFD and DD.

5.3 – Experimental

CFD fabrication

Channel fabrication

First, a hard mask of ~20 nm Al_2O_3 was deposited on silicon wafers (p-type, 100mm, 300-500 μm thickness, 0.01-20 Ω resistivity) using ALD (FlexAL Atomic Layer Deposition System, Oxford Instruments). This was followed by spin coating a double layer resist (P-20 resist overcoated with positive tone photoresist SPR 955CM-2.1, Microchem Corp.) and baking at 115°C for 90 seconds. Using a MA6/BA6 mask aligner (Süss MicroTech SE.), the wafer was exposed to UV light through a mask designed with channels using CAD software, after which the wafer was baked again for 90 seconds at 115°C and developed in CD-26 for 1 minute. Channels were subsequently etched on a Plasmalab 100 RIE/ICP Etcher (Oxford Instruments) using Bosch, sputter etch, and/or cryo etching processes to reach the desired channel's shape and depth.

PSO deposition and dicing

Plasma-enhanced chemical vapor deposition was used to deposit porous silicon oxide (PSO) on the wafer after the channels were created.[11-14] The 1 μm layer of PSO was deposited using a PECVD System 100 Plasma Deposition Tool (Oxford Instruments). The

deposition was carried out at a chamber pressure of 600 mTorr and substrate temperature of 27 °C. After PSO deposition the channeled wafer was diced into 1" × 1" substrates using a dicing saw (Accretech).

Nanoscribe

The features were created using COMSOL software (COMSOL, Inc.) and saved in a STL format. The files were transferred to the nanoscribe computer and converted using DeScribe (Nanoscribe GmbH) to GWL scripts followed by fabrication of the feature using a Nanoscribe GT Pro (Nanoscribe GmbH). The excitation source was an 800-nm femtosecond laser focused with a 25x or 63x microscope objective. Photoresist (IP-S or IP-Dip, Nanoscribe GmbH) was applied to the silicon substrate and placed in the sample holder. After exposure, development was carried out using 1-methyloxy-2-propanol acetate (SU-8 developer, Microchem Corp.) for 20 minutes then rinsed with 2-propanol (J.T. Baker) and dried under a stream of nitrogen. The features created using the 25x microscope objective and IP-S photoresist consisted of the side blocks and top. The mesh filters were printed using the 63x microscope objective and IP-Dip photoresist. The process flow is illustrated in Figure 5.3.1 and a cartoon of the final device is illustrated in Figure 5.3.2A.

Surface modification

After development, the substrates for particle separation were coated with ~20 nm of SiO₂ by ALD (FlexAL Atomic Layer Deposition System, Oxford Instruments). The substrates used in diffusion experiments were additionally coated with a thin layer of gold by physical vapor deposition.

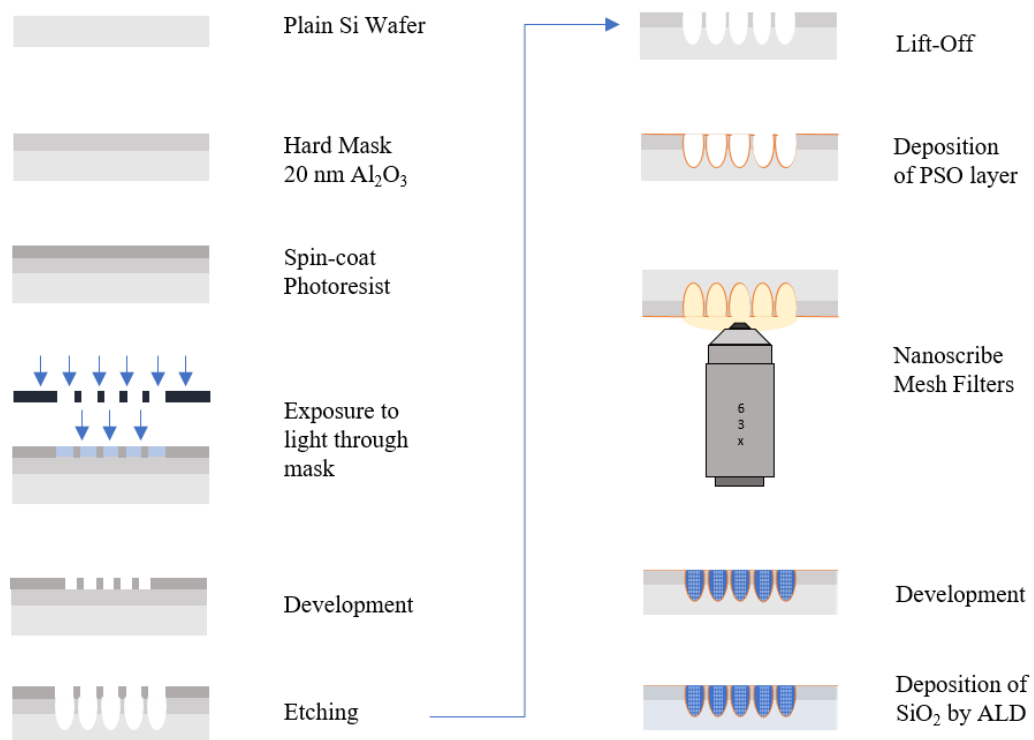


Figure 5.3.1: Fabrication process flow illustration for the fabrication of the capillary flow device.

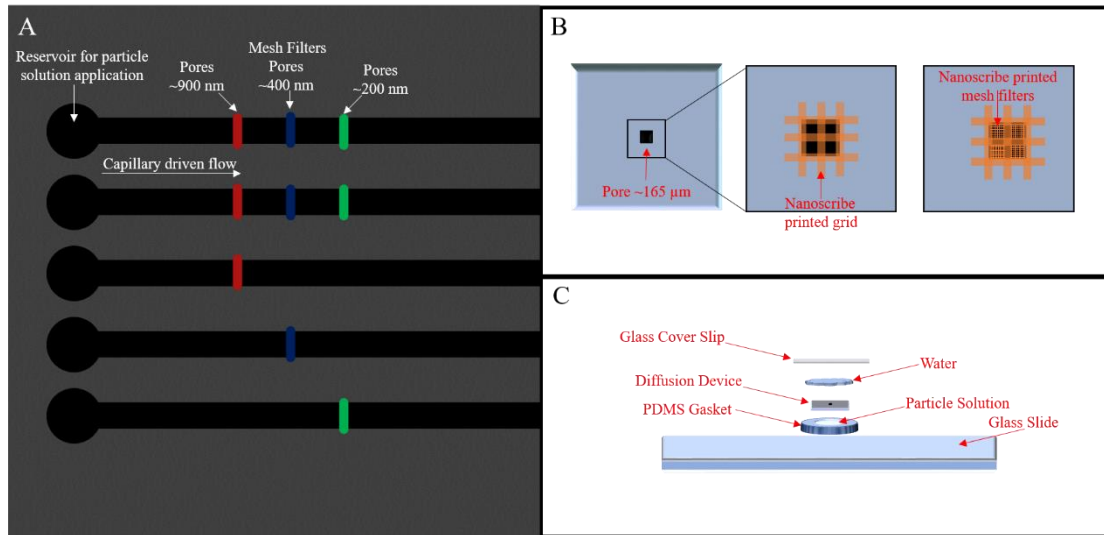


Figure 5.3.2: A is a cartoon illustration of the CFD highlighting the reservoir for sample application, placement and target pores sizes of the mesh filters, and the direction of capillary driven flow. B depicts the DD as it is printed: first the grid is printed over the pore in the chip, then the mesh filters are printed in the holes of the grid. C shows how the DD is set up for imaging of particle diffusion.

DD fabrication

The grid and mesh filters were designed using the same software and methods as described for the CFD. Printing of the DD was carried out on 1 × 1 cm chips with a 165 μm square hole in the center. The chip was mounted to the sample holder and photoresist (IP-Dip, Nanoscribe GmbH) was applied to the chip. The 63x microscope objective was used to focus the laser. After the grid and mesh filters were created, the chip was developed in 1-methyloxy-2-propanol acetate (SU-8 developer, Microchem Corp.) for 20 minutes, removed, and placed in a new solution of SU-8 developer for an additional 20 minutes. The chip was then rinsed with 2-propanol (J.T. Baker) and dried under a stream of nitrogen. Figure 5.3.3 shows the final design of the nanoscribe features for both devices.

CFD experiments

Deionized water was used to observe flow in the channels and through the meshes before particles were introduced. Particles (Fluoresbrite® BB Carboxylate Microspheres, Polysciences, Inc.) of sizes 300 nm, 500 nm, and 1.0 μm in deionized water were used to test the mesh filters. The particle solution (3×10^8 particles per milliliter) was applied to the reservoir at one end of the channel using a HPLC syringe and allowed to flow down the channels by capillary force. Detection of the particle separation was carried via fluorescence imaging using a Nikon Eclipse E600 microscope and Q-capture software.

DD experiments

The previously described particle solution was used for the both the DD and CFD experiments. A PDMS gasket was placed on top of a glass slide and filled with the particle solution. The DD was then set on top of the gasket, and a droplet of water was added to cover the pore of the DD. The experimental setup can be seen in Figure 5.3.2C. Images of the particles

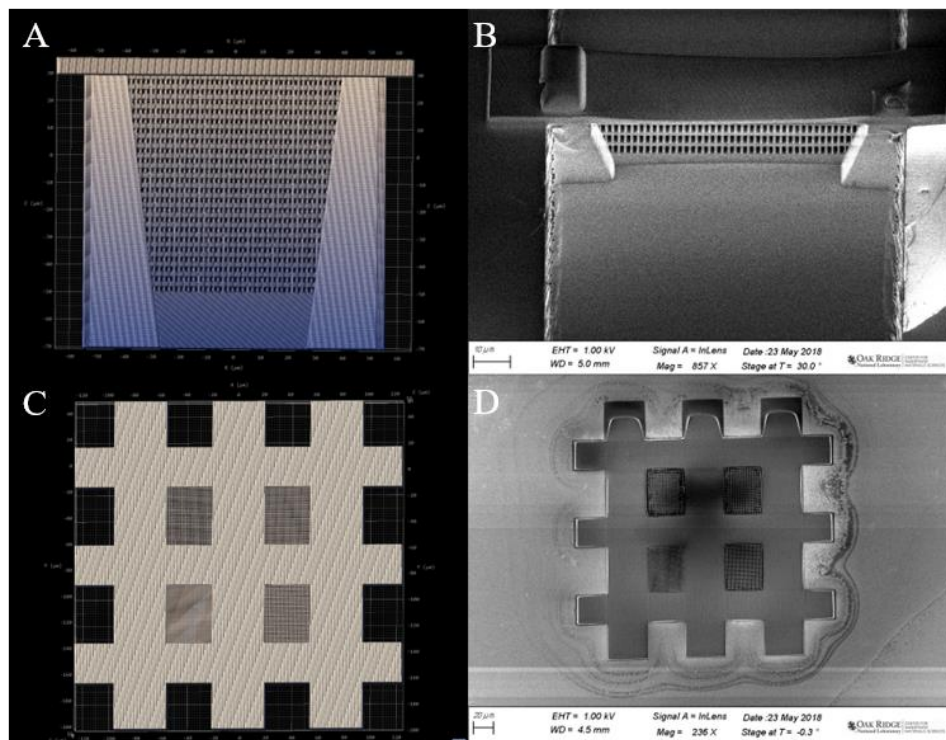


Figure 5.3.3: Computer generated image designs of the mesh filters for (A) the CFD and (C) the DD. (B) and (D) are the SEM images of the printed systems.

diffusing through the pore were captured using a fluorescent Nikon Eclipse E600 microscope with Q-capture software.

5.4 – Results and Discussion

CFD fabrication

Channels

In the first incarnation of the CFD platform mesh filters 5 μm thick and 40 μm wide were printed in channels ~ 25 μm wide. The challenges in this system included reproducibly applying the particle solution in the narrow channels, and the mesh filters not adhering to the channel and being displaced when the particle solution was applied. In the next version, new channels were fabricated with 100 μm widths. These larger widths allowed for increased ease and reproducibility of solution application. Additionally, the mesh filters were modified from being completely straight to having triangular a base and sides. This new mesh structure allowed for an increase of the surface area in contact with the bottom and sides of the channel. Finally, PSO was added to the channels prior to the nanoscribe printing. The high surface area of the PSO increased adhesion of the nanoscribe polymer to the channels, mitigating the problem of feature separation experienced previously.

Flow

There were two challenges observed when it came to flow. The first was that all particles passed through all meshes regardless of size. Upon examining the meshes with SEM imaging, it was determined that the bottom right side of the meshes were printing improperly and left a large gap negating any separatory value of the small pore meshes. This flaw in the mesh was due to shadowing in the bottom corner of the channel. The shadowing prevented the 63x objective from successfully printing in this region. However, it was found that with the use of a 25x objective

solid blocks could be printed in the shadow regions, effectively filling the space the 63x could not see. The polymer is transparent to the Nanoscribe instrument, allowing the mesh printed with the 63x to be printed into the block. Although this two-objective processing remedied the gap issue for a short time, in subsequent runs the problem became even more exaggerated. This increase in the shadowing issue was most likely due to either changes in laser power over time or a drifting instrument alignment, both of which create challenges in reproducible fabrication methods. To overcome these challenges, several iterations of side block design and printing methods were employed. First, slanted side blocks were used to compensate for the misprinting. However, as previously seen, the slanted blocks did not consistently print properly over time. Thus, a two-step angle block printing strategy was developed to help mitigate shadowing of the channel edge. This two-step strategy involved printing first on the right side of the channel, then removing and developing the blocks, followed by rotating the substrate 180° to print the blocks on the other side of the channel. This mid printing change in orientation allowed the objective to access the corner regions from the same incidence angle. Once again, the problem was only temporarily resolved and over time the persistent gap between the mesh and the printed block reappeared. Nanoscribe is a new technology, and problems that this work identified with drifting alignment or changes in laser power must be resolved if this technology is to be used to create reproducible separation platforms with a complex channel flow design. The various designs implemented to fix the problem can be seen in Figure 5.4.1.

Although a final reproducible fabrication method that addressed the mesh gap problems of the CFD platforms was not found, when the gap was temporarily fixed the usable platforms were tested for particle separation. During this work, it was found that the mesh filters were still not stopping the particles, and particles of all sizes would flow past the meshes. In order to

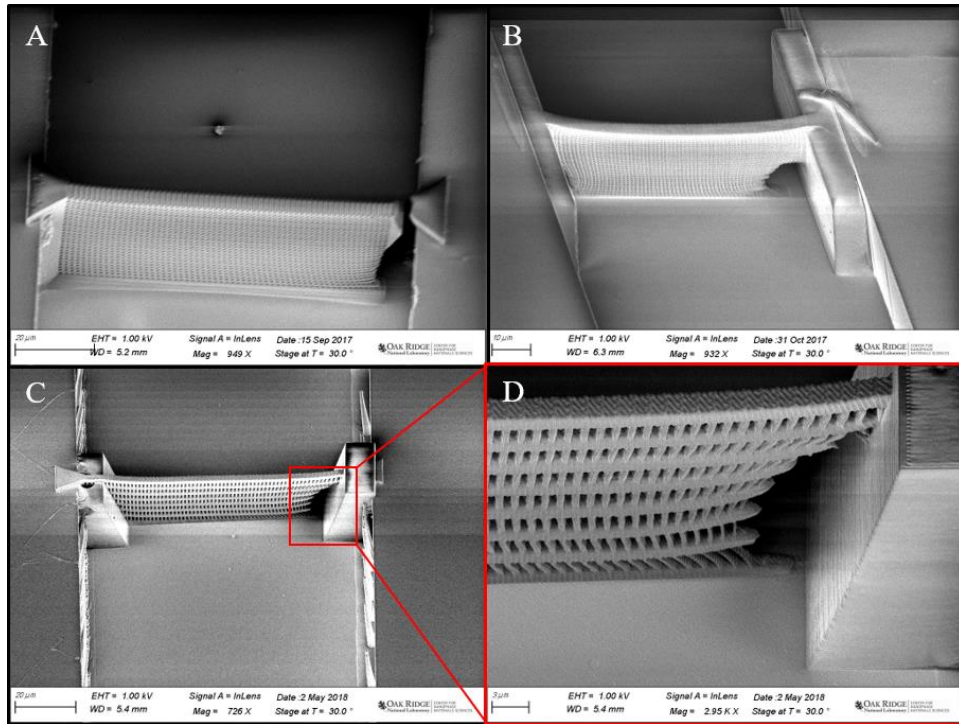


Figure 5.4.1: SEM images showing the shadowing affect: (A) original design of mesh when the problem was discovered, (B) side blocks added, (C) slanted side blocks, and (D) enlargement of the gap between the mesh and slanted side block.

diagnose this new problem, both fluorescent and SEM imaging were employed. Foremost, it was revealed that the flow was not staying within the channels. As the solution met the mesh filters, it would travel up the blocks around and over the top of the meshes instead of flowing through the separation pores. This flow path is evident in Figure 5.4.2. To address the solution confinement problem, a top was printed on the mesh filters to provide a physical barrier against overflow, as seen in Figure 5.3.3 A and B.

Another identified problem was pore occlusion. In experimentation, it was found that neither water nor particles would flow through the mesh filters. It was determined that the pores as originally designed were too small for the Nanoscribe to discretely print. After printing, the pores appeared open upon precursory evaluation; however, upon further examination it was determined they were not. The pore occlusion challenge was resolved by stretching the mesh in the X direction by a factor of 2, and shrinking the Y by a factor of 0.5. This change to design both increased the pore size and decreased the thickness of the mesh filters, resulting in pores large enough to discretely print and creating an open pore system traversing the entire thickness of the mesh. Subsequent experiments revealed that the chemical nature of the polymer would not allow water to easily flow through the open mesh filters. The hydrophobic nature of the polymer created a repulsive force that was not readily overcome by the capillary flow dynamics. This was mitigated by the deposition of a conformal layer of silicon dioxide (SiO_2), creating a hydrophilic surface that allowed water and particles to freely flow through the mesh filters. The evaluation of the SiO_2 coated mesh filters was promising. However, due to previously discussed fabrication challenges, the systems were not able to be reproducibly created for evaluation regarding particle separation in the systematic manner that they would require. Instead, due to the ever-drifting fabrication, a very limited number of potentially usable CFD systems were generated.

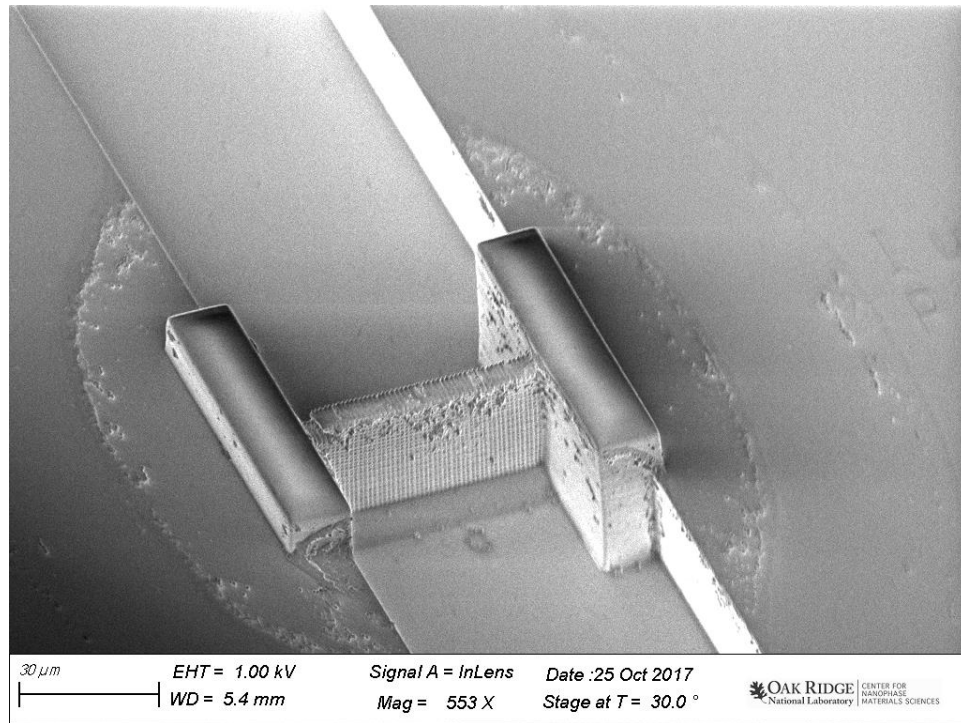


Figure 5.4.2: SEM image showing particles outside the channel flowing around and over the top of the mesh filter.

DD fabrication

The DD systems had similar fabrication challenges to the CFD systems. The two major challenges identified in the DD system included: 1) the pores in the mesh filters not being open all the way through and 2) the polymer of which the mesh filters were made being itself highly fluorescent. The pore occlusion was identified through SEM interrogation. As seen in the images Figure 5.4.3 of the printed diffusion system, the top of the filters (printed closer to the objective) appears to be open. However, evaluation of the bottom side of the system (printed further from the objective) revealed only a small number of the pores, mostly around the outer edges, traversed the entire system and most of the pores in the middle were occluded. This is an ongoing issue, with attempts to date unable to be resolved by changing the laser power or development procedure. Continued research into this problem is needed to find a solution. Secondly, the overly fluorescent nature of the polymer, which obscures particles diffusing through the mesh from being seen, needs to be addressed. The deposition of an alternative (potentially gold) metallic surface on the mesh, effectively blocking the absorption and emission of electromagnetic radiation from the mesh, should resolve the structure polymer background fluorescence.

CFD experiments

An attempt at particle separation using the CFD can be seen in Figures 5.4.4 and 5.4.5. It was designed so that ideally 1 μm particles would stop at the large mesh, 500 nm particles would stop at the medium mesh, and 300 nm particles would stop at the small mesh. Evaluation found that the 1 μm particles (that had a fluorescence emission in the red region) were almost completely retained by the large mesh filter. Additionally, the 500 nm particles (that had a fluorescence emission in the blue region) were indeed retained by the medium mesh filter as

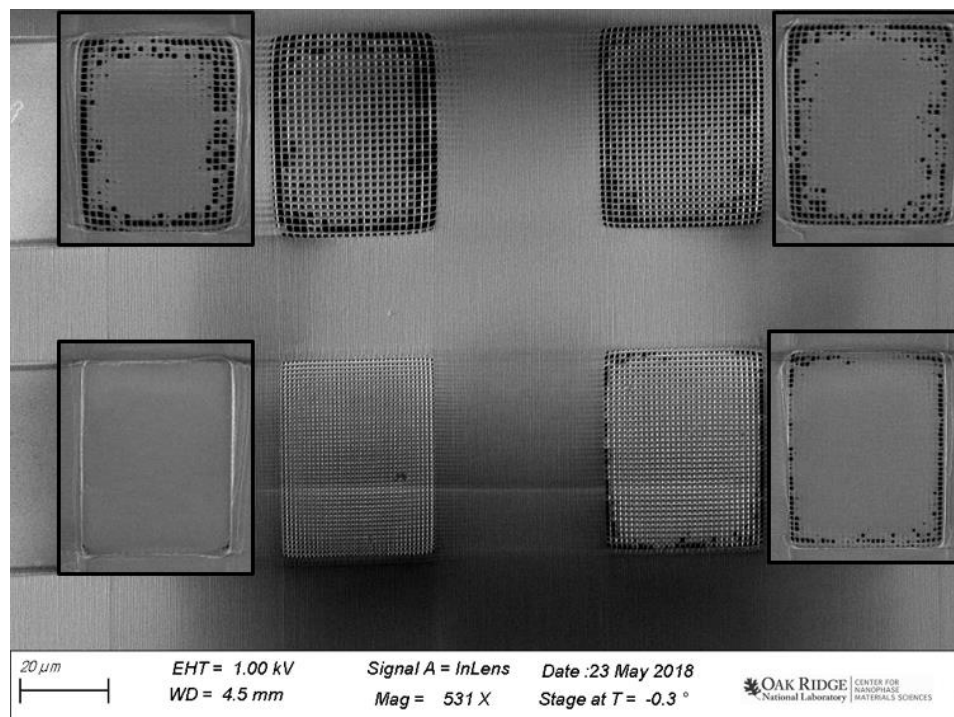


Figure 5.4.3: SEM images of the DD front (inner) and back (outer boxed) mesh filters, decreasing in size from right to left and top to bottom. Mesh filters imaged from the front appear to have open pores. However, images on the back clearly show the pores are mostly closed.

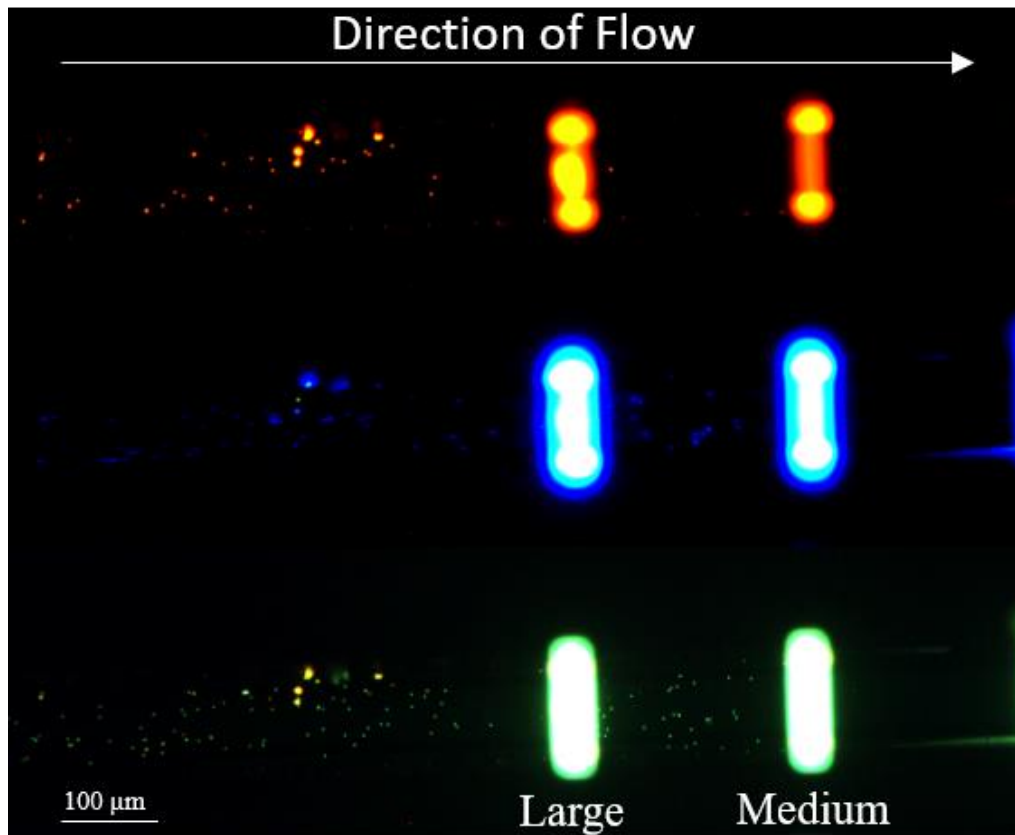


Figure 5.4.4: Fluorescent images of CFD particle separation attempt under different filters. The images from top to bottom show the 1 μm , 500 nm, and 300 nm particles. The 1 μm particle mostly did not pass through large mesh, while the 500 nm and 300 nm particles did not pass through the medium mesh.

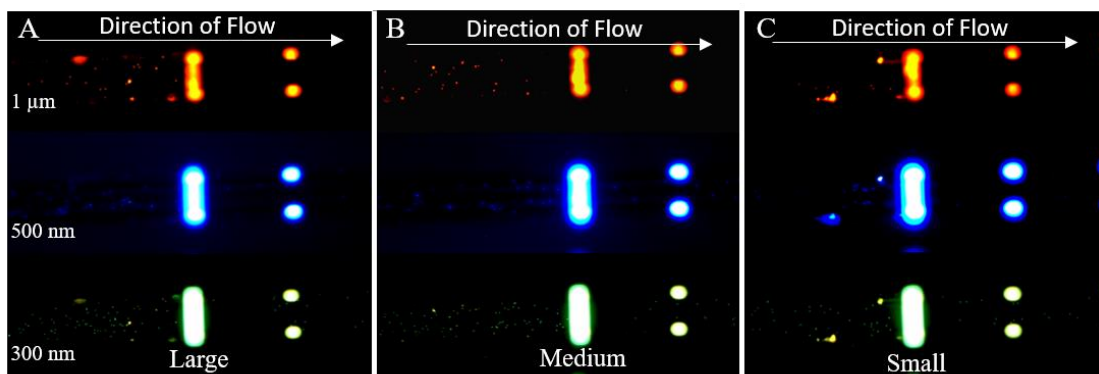


Figure 5.4.5: Fluorescent images of the CFD. (A) shows the 1 μm particles stopped at the large mesh while the 500 nm and 300 nm particles passed through. (B) shows the 1 μm particles stopped at the medium mesh while the 500 nm and 300 nm particles passed through. (C) shows the 1 μm and 500 nm particles stopped at the small mesh while the 300 nm particles passed through.

intended. However, the 300 nm particles (that had a fluorescence emission in the green region) were also retained by the medium filter, instead of passing through to be retained by the small mesh. Evaluation of the mesh, through SEM imaging, did not reveal that the medium mesh was occluded. In similar tests with another mesh printed at the same time, it was found that the smallest particles did indeed pass through the medium mesh. This inconstancy even among systems generated at the same time and under the same conditions highlights the fabrication challenges of reproducibly creating a separation system using the current Nanoscribe technology. Figure 5.4.5 shows the fluorescent images of particle in channels with large (A), medium (B), and small (C) mesh filters printed in the same run as those in Figure 5.4.4. In Figure 5.4.5 A and B, it is seen that only the 1 μm particles are stopped by the large and medium mesh filters while the 500 nm and 300 nm particles pass through. Figure 5.4.5 C shows that the 1 μm and 500 nm particles are stopped by the small mesh filter while the 300 nm particles were able to pass through. This is contradictory to what was seen in Figure 5.4.4, when the medium mesh stopped the 500 nm and 300 nm particles. Multiple variations of this were seen in other separation attempts in which mesh filters of the same size, printed in the same run, on the same substrate would not always stop particles of the same size.

DD experiments

An attempt at particle separation using the DD can be seen in Figures 5.4.6 and 5.4.7. Distinguishing any particles diffusing through the mesh filters in Figure 5.4.6 is nearly impossible due to the fluorescent background of the polymer. A few 1 μm particles (fluorescence emission in the red region) can be seen diffusing through the mesh. However, due to the fluorescence of the polymer, visualization of 500 nm particles (fluorescence emission in the blue region) and 300 nm particles (fluorescence emission in the green region) is not possible. Time

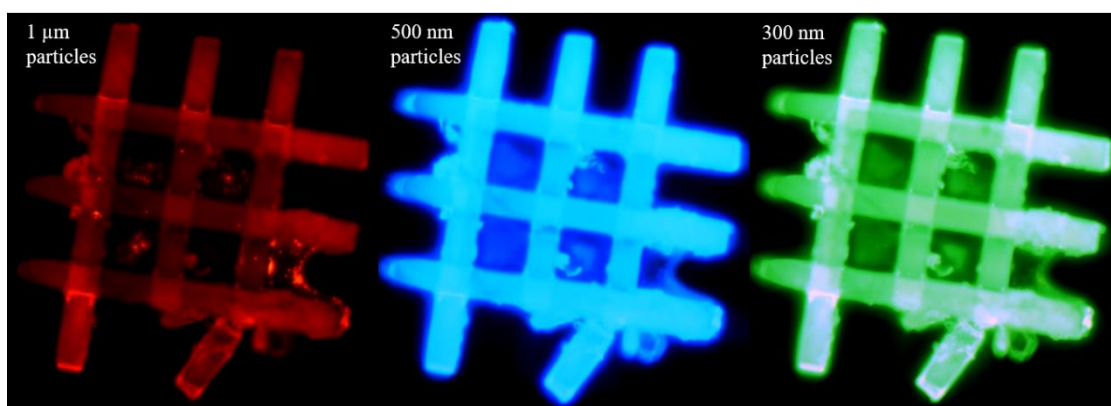


Figure 5.4.6: Fluorescent images of DD with particles.

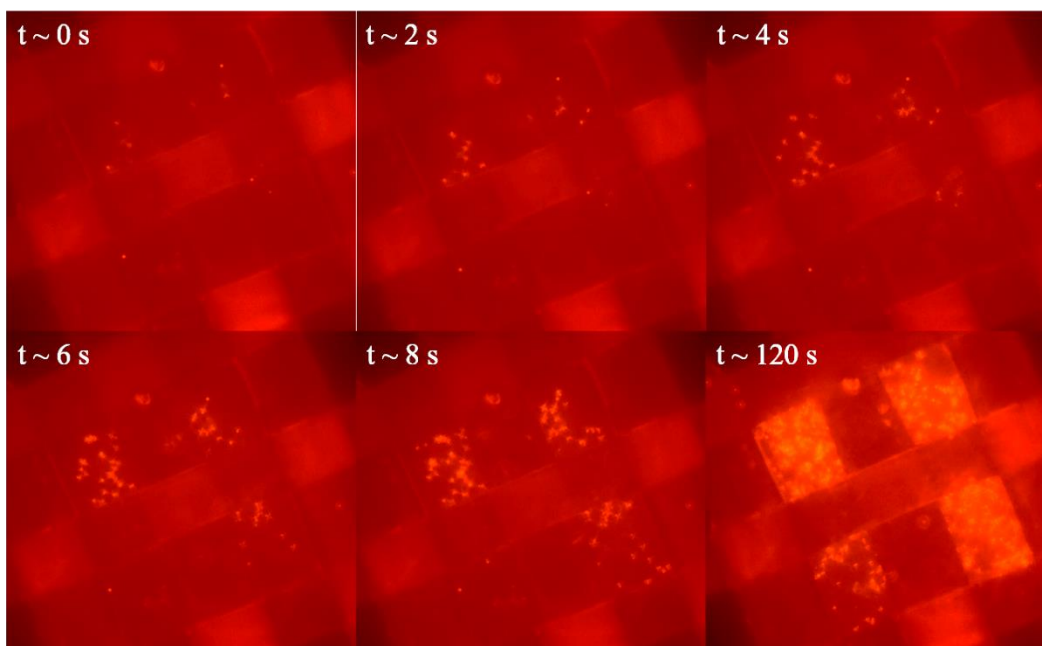


Figure 5.4.7: Time lapsed fluorescent images of $1 \mu\text{m}$ particles diffusing through the DD.

lapsed images of particle diffusion is seen in Figure 5.4.7, in which 1 μm particles can be seen diffusing through the pores of the mesh filters. It was intended that only the pores in the top left mesh filter be large enough for the 1 μm particle to pass through; however, the fluorescent images show that the particles were able to pass through all four of the mesh filters. Ideally, no particles would be retained by the top left filter, the 1 μm particles would be retained by the top right mesh filter, the 1 μm and 500 nm particle would be retained by the bottom right filter, and all particles would be retained by the bottom left filter. However, due to pore occlusion issues, fine tuning pore sizes to retain the desired particles was not pursued. The DD in Figure 5.4.7 was not free from occlusion issues as depicted in SEM images of the front and back of the device Figure 5.4.8. Current inconsistencies in the fabrication of the DD do not allow for successful use of the device for particle separation.

5.5 – Conclusions

The mesh filters created in this work are potentially tunable and can be fabricated to separate particles from a few hundred nanometers to several microns. Additionally, the positions of the meshes within the channels are adjustable, potentially allowing for separation of multiple sizes of particles to take place in areas ranging from less than a millimeter to over several centimeters. However, instability in the current Nanoscribe technology makes the reproducible fabrication, and therefore separation of particles, a near impossibility. Further advances in this fabrication technology and corresponding production methods could be vastly rewarding, allowing for complex separation combined with sample detection and chemical analysis to be carried out at the mesh filters. While the Nanoscribe technology offers the potential for creating these useful porous substrates, the technology is in its infancy and vastly more work will need to be conducted to address the challenges identified in this work.

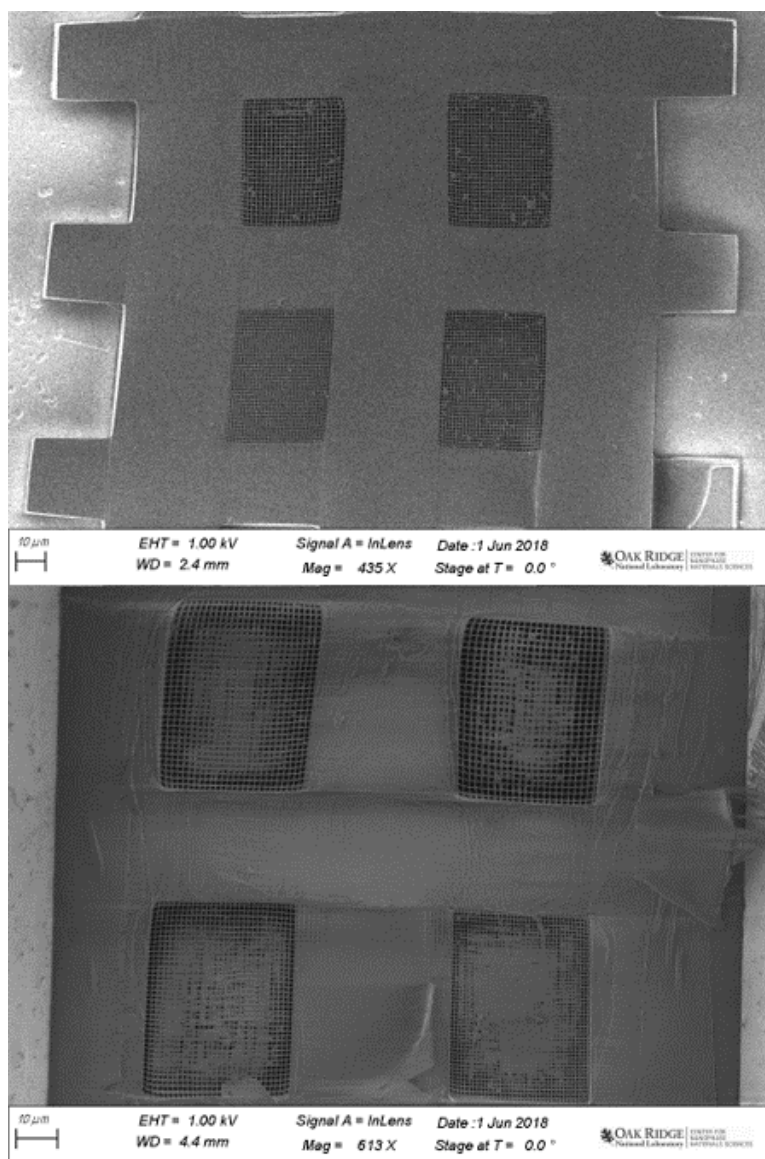


Figure 5.4.8: SEM images of the front and back of the DD from Figure 5.4.7.

5.6 – References

- [1] Roda, B., Zattoni, A., Reschiglian, P., Moon, M. H., Mirasoli, M., Michelini, E., Roda, A., *Anal. Chim. Acta* 2009, 635, 132-143.
- [2] Striegel, A. M., Brewer, A. K., in: Cooks, R. G., Yeung, E. S. (Eds.), *Annual Review of Analytical Chemistry, Vol 5*, Annual Reviews, Palo Alto 2012, pp. 15-34.
- [3] Salafi, T., Zeming, K. K., Zhang, Y., *Lab on a Chip* 2017, 17, 11-33.
- [4] Li, M., Li, W. H., Zhang, J., Alici, G., Wen, W., *Journal of Physics D-Applied Physics* 2014, 47.
- [5] Sajeesh, P., Sen, A. K., *Microfluid. Nanofluid.* 2014, 17, 1-52.
- [6] Ostasevicius, V., Golinka, E., Jurenas, V., Gaidys, R., *Mechanika* 2017, 23, 408-411.
- [7] Zhang, J., Yan, S., Sluyter, R., Li, W. H., Alici, G., Nguyen, N. T., *Sci Rep* 2014, 4.
- [8] Amato, L., Gu, Y., Bellini, N., Eaton, S. M., Cerullo, G., Osellame, R., *Lab on a Chip* 2012, 12, 1135-1142.
- [9] VanDelinder, V., Groisman, A., *Anal. Chem.* 2006, 78, 3765-3771.
- [10] Pinto, J., Dumon, M., Rodriguez-Perez, M. A., Garcia, R., Dietz, C., *Journal of Physical Chemistry C* 2014, 118, 4656-4663.
- [11] Wallace, R. A., Sepaniak, M. J., Lavrik, N. V., Datskos, P. G., *Anal. Chem.* 2017, 89, 6273-6277.
- [12] Strickhouser, R. B., Hatab, N. A., Lavrik, N. V., Sepaniak, M. J., *ELECTROPHORESIS* 2018, 39, 438-444.
- [13] Lincoln, D. R., Lavrik, N. V., Kravchenko, I. I., Sepaniak, M. J., *Anal. Chem.* 2016, 88, 8741-8748.

[14] Charlton, J. J., Lavrik, N., Bradshaw, J. A., Sepaniak, M. J., *ACS Applied Materials & Interfaces* 2014, 6, 17894-17901.

Chapter 6

Summary

6.1– Centrifugal-Driven, Reduced-Dimension, Planar Chromatography Systems

Based on chromatographic theory, the mobile phase velocity is slower than the optimum velocity, therefore efficiency suffers. Efficiency is limited by the diminishing flow rates as development proceeds via capillary action, thus resulting in band dispersion. The work described herein aimed to improve the efficiency of micro- and nanopillar array planar chromatography systems fabricated using photolithography and metal dewetting by increasing the mobile phase velocity using centrifugal force.

Preliminary experiments resulted in separations resulting from two different solvent flow patterns: complete-flow – the solvent radiating from the center to the edges of the substrate in all directions – and creek-like flow – the solvent traveling from the center to the edge of the in one or more discrete narrow creeks. While both types of flow were observed, the creek-like flow was far more prevalent. The centrifugal driving force of these separations was calculated to be less than the resistive force within the pillar arrays, and yet separation was achieved; this was due to the co-planar flow driving the flow within the pillars, which rapidly drives development.

The main challenge with this system was the random nature of the creek-like flow pattern. Therefore to improve reproducibility, channels were employed to confine the solvent flow. However, channels etched into the substrate either did not sufficiently confine the solvent, or the solvent would flow along the walls of the channels thus prohibiting separations; channels fabricated on top of the pillar arrays out of PDMS or SU-8 were also unsuccessful at confining flow due to improper bonding and channel distortions. Attempts to confine solvent flow were abandoned and efforts were made to instead direct solvent flow through the use of central features created by a Nanoscribe 3D micro-printing system (Nanoscribe GmbH). High speed video revealed one such fabricated central feature produced the desired narrow creek-like solvent

flow pattern. In continuation of this research, additional substrates need to be fabricated with the central feature, studies need to be conducted to determine the proper solvent volume and dispense rate required to reproducibly produce the narrow creek-like flow pattern, and finally, separation experiments need to be run.

6.2– Nanoscribe Printed Mesh Filters for Particle Separations

The ability to separate particles is useful in chemical, biological, and medical fields. Flow fractionation, electrophoresis, and hydrodynamic filtration are a few of the various techniques used for large scale particle separations. More recently, LOC devices have been used to achieve particle separations due to their use of small sample volumes, portability, and rapid analysis. The work presented here used multi-method LOC processes photolithography and reactive ion etching combined with 3D micro printing via 2PP using a Nanoscribe (Nanoscribe GmbH) to create devices aimed at separating high-value samples of micro- to nanoparticles in low volumes (< 100 nL). Two separation devices were created, a capillary action device and a diffusion device. Both devices employed mesh filters, fabricated with a variety of pores sizes to separate particles ranging in size from 1 μm to 300 nm.

Pore occlusion, flow confinement, and shadowing were the main challenges experienced during the fabrication process of the CFD. The pore occlusion problem was resolved by stretching the mesh filters in the Z direction thus elongating the pores to twice the height, and shrinking filters in the Y direction to make the filters half as thick. Flow confinement was achieved by printing a top on the mesh filters. Blocks, and then slanted blocks, were printed along the walls of the channels to compensate for the reoccurring shadowing problem that left a gap in the mesh next to the channel wall on one side. However, the shadowing became more exaggerated due to the instrument's drifting alignment or changing laser power.

Pore occlusion and the fluorescent nature of the polymer of which the mesh filters were made were the main challenges of the DD. The pores were open on one side of the mesh filter but blocked on the other. Development times were extended and the laser power of the instrument was adjusted during printing in an effort to resolve the pore occlusion on the backside of the mesh filters. However, neither adjustment was successful. In order for this project to move forward, the problems such as the misalignment and decreasing laser power of the Nanoscribe instrument need to be resolved; in addition, the surface of the DD needs to be modified by depositing a metallic layer to decrease the fluorescence of the mesh filter polymer, to allow particle diffusion to be visualized.

Vita

Rachel Brooke Strickhouser was born in South Carolina to Doug and Jill Strickhouser in 1989. She was raised in Lexington, South Carolina where she attended high school, graduating from Lexington High School in 2007. She continued her education at the University of South Carolina Aiken performing research into the development of new sensor substrates for surface enhanced vibrational spectroscopy under the mentorship of Dr. Chad Leverette. In 2012, Rachel earned her Bachelors of Science in Chemistry. Upon graduation Rachel continued research with Dr. Leverette for a year. She then pursued her Doctorate of Philosophy in Analytical Chemistry at the University of Tennessee Knoxville under Dr. Michael J. Sepaniak. She will graduate in Summer 2018 and continue her academic career with a teaching postdoctoral position at the University of South Carolina Aiken.